MAJOR ARTICLE

Superantigen Profile of *Staphylococcus aureus* Isolates from Patients with Steroid-Resistant Atopic Dermatitis

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Background. Superantigens induce skin inflammatory responses in atopic dermatitis, which is commonly associated with *Staphylococcus aureus* infection. T cells activated in vitro by superantigens become steroid resistant. The objective was to assess the superantigen profiles of *S. aureus* isolates from patients with steroid-resistant atopic dermatitis.

Methods. We compared the superantigen-production capability of *S. aureus* isolates from 78 patients with steroid-resistant atopic dermatitis (group 1) with that of 30 vaginal isolates from healthy women (group 2) and 22 isolates from a general population of patients with atopic dermatitis (group 3). Polymerase chain reaction with primers for superantigens, combined with selected antibody testing, was used to analyze the presence of toxic shock syndrome toxin 1, staphylococcal enterotoxins, and enterotoxin-like superantigens.

Results. *S. aureus* isolates from group 1 had a statistically significant difference in superantigen profile, compared with the profiles of group 2 and group 3 isolates. Group 2 isolates were similar in profile to group 3 isolates, with 4 and 5 superantigens per isolate, respectively. In contrast, group 1 isolates produced a mean of 8 superantigens each ($P \ll .001$, for comparison with group 2 or group 3). These group 1 isolates were more likely to produce the 3 major toxic shock syndrome–associated superantigens (toxic shock syndrome toxin 1, staphylococcal enterotoxin B, and staphylococcal enterotoxin C) and to produce unusual combinations of superantigens (e.g., toxic shock syndrome toxin 1 and staphylococcal enterotoxin B).

Conclusions. *S. aureus* isolates from patients with steroid-resistant atopic dermatitis appear to be selected on the basis of greater production of superantigens, compared with that of isolates from control groups. Superantigens may offer selective advantages for colonization of patients.

Staphylococcus aureus is a commensal organism that colonizes up to 50% of humans [1, 2]. The organism most often colonizes the anterior nares and, from there, may colonize other body surfaces, including other mucous membranes and damaged skin. *S. aureus* causes a wide variety of human illnesses, including scalded skin syndrome, toxic shock syndrome (TSS), and necrotizing pneumonia [3–9].

The ability of *S. aureus* to cause human disease depends on the production of cell-surface adhesins, antiphagocytic factors, and secreted exotoxins, whose functions appear to be both securing nutrients for the microbes and delaying function of the immune system [10–12]. Among the secreted factors is a large family of superantigen exotoxins [8].

Staphylococcal superantigens include staphylococcal enterotoxins, classically the common causes of food poisoning and nonmenstrual TSS, and TSS toxin 1 (TSST-1), the cause of both menstrual and nonmenstrual TSS [8]. Staphylococcal enterotoxin serotypes A–E (SEA–SEE) and SEG–SEQ have been well described in the literature. SEA–SEE and SEI are capable of causing vomiting and diarrhea when administered to monkeys and, thus, are correctly referred to as staphylococcal enterotoxins [13]. The remaining staphylococcal enterotoxins either lack emetic activity (SEG, SEK, SEL, and SEQ) or have not been tested for emetic activity. According to the suggestions of a recent nomenclature

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committee, these superantigens are more correctly designated as staphylococcal enterotoxin–like (SEl) (SEl-G, -H, -J, -K, -L, -M, -N, -O, -P, and -Q) [14].

Superantigens are defined by their ability to stimulate cytokine release from both T cells and macrophages [15]. The proteins bind to relatively invariant regions of major histocompatibility complex II molecules on antigen-presenting cells, and they cross-bridge with certain variable regions of the beta-chains of T cell receptors (Vβ-TCR) [16]. Each superantigen has a relatively unique subset of Vβ-TCR interactions. For example, TSST-1 stimulates only T cells bearing Vβ2-TCRs, but these account for only ~10% of the total repertoire of T cells in humans [16]. However, during acute TSS, T cells bearing Vβ2-TCRs may proliferate in a skewed manner, such that the activated T cells account for 60% of the patients' T cells [17]. The massive cytokine release by both T cells and macrophages accounts for the most-severe manifestations of superantigen-mediated illnesses [8, 15, 16].

Atopic dermatitis is a T cell–mediated skin disease that can significantly compromise quality of life, because patients experience sleep disturbances, social embarrassment, and emotional distress. *S. aureus* infection contributes to the worsening of skin inflammation in atopic dermatitis [18–20]. These organisms have been shown to produce superantigens, including SEA, SEB, SEC, and TSST-1. However, the full spectrum of superantigens produced by *S. aureus* isolates that infect patients with atopic dermatitis has not been examined previously. Furthermore, superantigens have been demonstrated to induce corticosteroid resistance of T cells in vitro [21]. This could contribute to difficulty in management of atopic dermatitis, because topical corticosteroids are the most common medication used for treatment of atopic dermatitis. The present study, therefore, was undertaken to characterize *S. aureus* isolates from patients with steroid-resistant atopic dermatitis with regard to their ability to produce superantigens.

MATERIALS AND METHODS

S. aureus *isolates.* Three groups of isolates were compared for the presence of superantigen genes. Group 1 isolates included 78 isolates from patients with steroid-resistant atopic dermatitis. Steroid resistance was defined by a <35% reduction in the patient's eczema area and severity index during treatment with a topical steroid, prednicarbate emollient cream, twice a day for at least 12 days. Cotton swabs were used to obtain individual samples of the 4 most-affected eczematous lesions, which were evaluated for the growth of *S. aureus* colonies. *S. aureus* isolates were identified by colony morphology (gram-positive, cluster-forming cocci), positive catalase activity, and positive coagulase activity. Group 2 isolates for comparison included 30 vaginal isolates from healthy women randomly collected in 2003–2005 and previously published [22]. The 22 group 3 isolates were obtained in 2002 from a general population of patients with atopic dermatitis, irrespective of responsiveness to treatment.

PCR for superantigen genes and assays for exotoxins. For superantigen gene testing, all organisms were cultured at 37°C with shaking (at 200 rpm) in 10 mL of Todd Hewitt broth and then were used for DNA extraction [22]. The primers used for PCR amplification of superantigen genes were published previously [22]. The conditions for PCR were described in detail elsewhere [22].

For use in quantification of selected superantigens, organisms were cultured in a dialyzable beef heart medium (25 mL in 125-mL Erlenmeyer flasks) at 37°C with shaking (at 200 rpm) [23]; the superantigens included TSST-1, SEA, SEB, and SEC. Superantigens in these samples were concentrated 10–100-fold by consecutive precipitation with the addition of 4 volumes of absolute ethanol for 2 h, centrifugation at 4000 *g* for 10 min, and resolubilization in distilled water [23]. Superantigens were quantified by antibody assays (Western immunoblotting) with use of polyclonal rabbit antisera raised against the individual superantigens [24]. In brief, known quantities of purified superantigen standards (0.1, 0.01, and 0.001 ug/mL, as purified in the laboratory of P.M.S.) and concentrated culture fluids were electrophoresed in sodium dodecyl sulfate on 10% polyacrylamide gels and were blotted onto polyvinylidene fluoride membranes (Bio-Rad Laboratories). Subsequently, the membranes were blocked with 1% albumin, were incubated for 2 h with polyclonal rabbit antisera (raised in the laboratory of P.M.S. or provided by Toxin Technology) to the individual superantigens, were washed to remove unbound antisera, were incubated for 2 h with alkaline phosphatase–conjugated anti-rabbit IgG (whole molecule; Sigma Aldrich), were washed to remove unbound conjugate, and were developed with substrate. The densities of band on the developed immunoblots were determined with use of a computer program provided by the National Institutes of Health (ImageJ, version 1.34S) [25]. The lower limit of toxin detected by this method was 1.0 ng/mL of sample (0.01 pg/mL in 100-fold concentrated culture fluids).

***Sequencing studies of* srrA-srrB.** During the course of evaluation of superantigen production by *S. aureus* isolates from patients with steroid-resistant atopic dermatitis, it became clear that superantigen-production profiles and the amounts produced appeared to be dysregulated. Thus, we evaluated the 2-component regulatory system of staphylococcal respiratory response (SrrA-SrrB) for altered regulation of superantigen production. SrrA-SrrB represses exotoxin production under low-oxygen conditions, loses repression activity when oxygen levels exceed 2%, and appears to regulate other critical global regulatory systems, such as the accessory gene regulator [26, 27]. The *srrA-srrB* locus was sequenced in 6 isolates from patients

Table 1. Superantigen genes in *Staphylococcus aureus* isolates from patients with steroid-resistant atopic dermatitis (AD) (group 1), vaginal isolates from healthy women (group 2), and isolates from a general population of patients with AD (group 3).

Superantigen	Group 1 ($n = 78$)	Group 2 ($n = 30$)	Group 3 ($n = 22$)	P[a]	
				Group 1 vs. group 2	Group 1 vs. group 3
SEA	37 (47)	8 (27)	4 (18)	.05	.015
SEB	33 (42)	3 (10)	2 (9)	.001	.005
SEC	23 (29)	9 (30)	2 (9)	NS	NS
SED	38 (49)	4 (13)	1 (4.5)	⩽.001	.001
SEE	33 (42)	6 (20)	6 (27)	.04	NS
SEI-G	44 (56)	9 (30)	11 (50)	.02	NS
SEI-H	43 (55)	3 (10)	0 (0)	⩽.001	⩽.001
SEI	38 (49)	10 (33)	10 (45)	NS	NS
SEI-J	67 (86)	8 (27)	2 (9)	⩽.001	⩽.001
SEI-K	45 (58)	23 (77)	4 (18)	NS	.001
SEI-L	21 (27)	29 (97)	8 (36)	⩽.001	NS
SEI-M	55 (71)	7 (23)	9 (41)	⩽.001	.02
SEI-N	50 (64)	5 (17)	1 (4.5)	⩽.001	⩽.001
SEI-O	33 (42)	2 (7)	4 (18)	⩽.001	.05
SEI-Q	31 (40)	18 (60)	9 (41)	NS	NS
TSST-1	27 (35)	12 (40)	11 (50)	NS	NS

NOTE. Data are no. (%) of isolates positive for the specified superantigen gene, unless otherwise indicated. NS, not significant; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1.

[a] By Fisher's exact test.

with steroid-resistant atopic dermatitis, to assess the function of this regulatory system [28].

RESULTS

We compared, by PCR, the superantigen profiles of 2 groups of isolates: 30 previously published vaginal isolates from healthy women [22] and 22 isolates from a general population of patients with atopic dermatitis. We evaluated the capacity of isolates to produce TSST-1, SEA–SEE, SEI, SEl-G, SEl-H, SEl-L, SEl-M, SEl-N, SEl-O, and SEl-Q by PCR for the respective genes (table 1); the nucleotide sequence of the *sel-P* gene became available only when this study was nearly completed; thus, the superantigen SEl-P was omitted from the study. Several important observations were made relative to the 3 groups of isolates. The capacity of group 1 isolates from steroid-resistant patients to produce superantigens differed significantly from that of group 2 isolates (vaginal isolates from healthy women) and group 3 isolates (from a general population of patients with atopic dermatitis) (table 1). Group 1 isolates were significantly more likely to have the genes for SEA, SEB, SED, SEl-H, SEl-J, SEl-M, SEl-N, and SEl-O than were both group 2 and group 3 isolates. Group 1 isolates also were more likely than group 2 isolates to have the genes for SEE, SEG, and SEl-L and were more likely than group 3 isolates to have the gene for SEl-K. There was no statistically significant difference among the 3 groups for the genes for SEC, SEI, SEl-Q, and TSST-1. The group 2 isolates were not significantly different in superantigen profile than the group 3 isolates; with the exception that group 2 isolates had the genes for SEl-K and SEl-L significantly more often than did group 3 isolates ($P \ll .001$). As shown in table 2, the group 1 isolates produced a mean of 8 superantigens; only 1 isolate did not make any known superantigens, and 1 isolate had the genes for all known superantigens except TSST-1. In contrast, the group 2 and group 3 isolates produced a mean of 5 and 4 superantigens, respectively (the difference was not statistically significantly), both of which were significantly less than that produced by group 1 isolates. One group 2 isolate and 3 isolates from group 3 were negative for known superantigens.

Several other interesting observations were made concerning the group 1 isolates (table 3). SEl-G, SEl-M, SEl-N, SEl-O, and SEI are thought to be linked, in order, as an enterotoxin gene cluster [29]. Of the group 1 isolates, only 19 (24%) contained the intact cluster. In contrast, 63 (81%) of the isolates contained the genes for at least 1 superantigen in the cluster, suggesting that the enterotoxin gene cluster is not intact in the majority of these isolates. In tests of >5000 TSS isolates, only 2 have been shown (by the laboratory of P.M.S.) to produce the com-

Table 2. Comparison of superantigen production among *Staphylococcus aureus* isolates from patients with atopic dermatitis (AD) and vaginal *S. aureus* isolates.

S. aureus isolates	No. of superantigens, mean ± SD	P[a] for comparison with		
		Group 1	Group 2	Group 3
Group 1	8 ± 3	…	…	…
Group 2	5 ± 1.7	⩽.001	…	NS
Group 3	4 ± 3	⩽.001	…	…

NOTE. Group 1 are isolates from patients with steroid-resistant AD, group 2 are vaginal isolates from healthy women, and group 3 are isolates from a general population of patients with AD. NS, not significant.

[a] By Student's *t* test.

bination of SEB and SEC. Of the 78 group 1 isolates, 12 (15%) were positive for genes for both these toxins, compared with 2 (3.8%) of 52 isolates from groups 2 and 3 combined. Likewise, it is generally thought that *S. aureus* strains cannot produce SEB and TSST-1 simultaneously [30–32]. However, 8 (10%) of the group 1 isolates were positive for both these toxins. Approximately 15% of *S. aureus* isolates from patients with TSS have the genes for SEC and TSST-1 together [33]; there was a similar rate of 12% among the group 1 isolates (9 of 78 were positive). The 3 major causes of staphylococcal TSS are TSST-1, SEB, and SEC [8, 32]. Of the group 1 isolates, 57 (73%) were positive for ⩾1 of these toxins.

TSST-1, SEB, and SEC were quantified after in vitro culture to evaluate potential differences in the quantity of these superantigens produced by the group 1, group 2, and group 3 isolates. There were no differences among the groups in the amounts of superantigens produced in culture media; the range of values was 3–20 ug/mL for TSST-1, 25–80 ug/mL for SEB, and 40–80 ug/mL for SEC. Interestingly, in the course of these studies, it appeared that group 3 isolates produced more SEA (4–5 ug/mL) than did typical TSS isolates (typically 0.01 ug/mL). In light of this observation, 3 of the group 1 isolates (including the isolate that contained the genes for all known superantigens except TSST-1) were compared with 3 menstrual TSS isolates for growth and superantigen-production characteristics, particularly for SEA (figure 1). The menstrual TSS isolates grew to slightly higher cell densities in the 12-h test period, compared with the group 1 isolates. All 3 of the menstrual TSS isolates produced both TSST-1 and SEA, with TSST-1 produced primarily in the postexponential phase and with SEA produced primarily during exponential growth. These data are consistent with those of previous studies [10, 34, 35]. In contrast, the 3 atopic dermatitis isolates produced SEB and SEC at high concentrations in the postexponential phase, as expected, but the isolates produced SEA in higher concentrations than did the menstrual TSS isolates (10 ug/mL vs. 0.1 ug/mL), and SEA was produced predominantly during the postexponential phase. These studies suggested that the group 1 isolates not only produced unusual combinations of superantigens (table 3) but also dysregulated SEA production, compared with other isolates.

SrrA-SrrB is a global, 2-component system regulator of exotoxin production in *S. aureus* [26, 27]. This system represses superantigen production in low-oxygen conditions (oxygen level, <2%); its activity is lost in the presence of oxygen [26, 27]. The 2-component system was sequenced in 6 group 1 isolates; of the 6 isolates tested, 2 were defective in their ability to produce Srr because of truncations in the *srrA-srrB* locus [28].

DISCUSSION

S. aureus superantigens play an important role in the natural course of atopic dermatitis [18–20]. Previous studies have focused on only a few superantigens produced by *S. aureus* strains obtained from patients with atopic dermatitis. The present study is the most comprehensive study of superantigens in patients with atopic dermatitis, examining all well-characterized superantigens except SEl-P, which is an uncommon superantigen.

Several important observations were made. First, the *S. aureus* strains from patients with steroid-resistant atopic dermatitis showed the ability to produce large numbers of superantigen types per organism, significantly higher than those produced by other skin isolates (group 3 isolates from a general population with atopic dermatitis) and by mucous membrane isolates (group 2 vaginal isolates). Each superantigen is known to activate only a subset of T cells expressing particular Vβ-TCR regions [15, 16]. The net effect of *S. aureus* strains producing a larger number of superantigens types would be to recruit larger numbers of T cells to produce proinflammatory cytokines and to induce a wider spectrum of T cells that fail to respond to the immunosuppressive effects of corticosteroids. This process could thus contribute to steroid-resistant atopic dermatitis.

Second, our studies suggest that the *S. aureus* isolates from

Table 3. Specific superantigen combinations in *Staphylococcus aureus* isolates from patients with steroid-resistant atopic dermatitis (group 1).

Superantigen combination	No. (%) of isolates positive
EGC[a]	19 (24)
SEB and SEC	12 (15)
SEB and TSST-1	8 (10)
SEC and TSST-1	9 (12)
Any 1 of SEB, SEC, or TSST-1	57 (73)

NOTE. EGC, enterotoxin gene cluster; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1.

[a] SEl-G, SEl-M, SEl-N, SEl-O, and SEl.

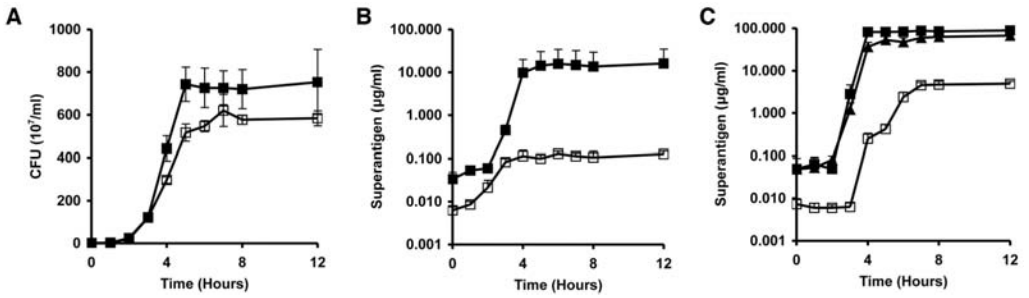

Figure 1. Growth of *Staphylococcus aureus* isolates and production of superantigens in vitro. *A,* Mean growth of 3 isolates from patients with menstrual toxic shock syndrome (TSS) *(blackened squares),* compared with the mean growth of 3 isolates from patients with steroid-resistant atopic dermatitis *(unblackened squares). B,* Mean production of TSS toxin 1 *(blackened squares)* and staphylococcal enterotoxin (SE) serotype A *(unblackened squares)* by the same 3 menstrual TSS isolates. *C,* Mean production of SEB *(blackened squares),* SEC *(blackened triangles),* and SEA *(unblackened squares)* by 3 isolates from patients with steroid-resistant atopic dermatitis. Bars represent SDs of the mean. CFU, colony-forming units.

patients with steroid-resistant atopic dermatitis have been selected for their production of greater numbers of superantigens than those produced by isolates from a general population of patients with atopic dermatitis and by vaginal isolates from normal healthy women. Although the cause of this selection is unknown, the use of steroids to manage atopic dermatitis, with the intent to reduce inflammation and consequent skin damage, would typically create cutaneous environments that reduce *S. aureus* colonization. Increased superantigen production by individual organisms may be necessary to promote skin inflammation and continued staphylococcal infection in patients with atopic dermatitis. Alternatively, the antimicrobial treatment approaches, including antibiotic treatment, used for management of atopic dermatitis should be investigated to determine their selection bias for such strains of *S. aureus.*

Third, in addition to having the potential to make more types of superantigens, *S. aureus* isolates from patients with steroid-resistant atopic dermatitis also have dysregulated production of superantigens and produce unusual combinations of superantigens. For example, it is commonly assumed that *S. aureus* strains cannot produce the superantigens TSST-1 and SEB together, possibly because the pathogenicity islands that encode the toxins may occupy the same chromosomal position [30–32]. This is clearly not the case, because several isolates from patients with steroid-resistant atopic dermatitis coproduced both superantigens. In addition, superantigen production is strictly under the control of the global regulator SrrA-SrrB [12, 27]. This 2-component system functions as a repressor of exotoxin production by *S. aureus* under low-oxygen conditions. Its repressive effects are lost in the presence of >2% oxygen [36]. Thus, no superantigens are produced by *S. aureus* under anaerobic conditions, despite the organism's ability to grow anaerobically. We observed that 2 of 6 *S. aureus* isolates had truncated genes for SrrA-SrrB. These strains are able to produce superantigens, even under unfavorable, low-oxygen conditions [28]. We showed that *S. aureus* isolates from patients with steroid-resistant atopic dermatitis are more likely than other *S. aureus* isolates to produce the superantigens typically made in high concentrations, such as TSST-1, SEB, and SEC. In addition, the organisms produce SEA in the postexponential phase (when bacterial cell densities are high) rather than in the exponential phase [10, 34], giving these organisms the ability to make higher concentrations of this superantigen than would be expected. Finally, our studies show that production of superantigens, such as the enterotoxin gene cluster [29] of proteins (SEl-G, SEl-M, SEl-N, SEl-O, and SEI), has been altered. In many strains, these superantigens are no longer linked on a single pathogenicity island.

Collectively, the present study suggests that *S. aureus* isolates from patients with steroid-resistant atopic dermatitis are being selected for their greater capability to produce superantigens. These proteins are critical virulence factors for *S. aureus* strains. The data suggest that the spread of such strains to other humans may predict their causation of additional illnesses. For example, a TSST-1–positive strain, without a functional SrrA-SrrB system, could cause menstrual, vaginal TSS without regard to the oxygen content of the vagina. It has been hypothesized that the association of tampons with TSS is related to their oxygenation of a typically anaerobic environment [37, 38]. In individuals with TSST-1–positive strains, TSS may occur in the absence of tampon use.

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References

1. Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R. Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992–2003. Clin Infect Dis **2006**; 42:389–91.
2. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med **1998**; 339: 520–32.
3. Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. MMWR Morb Mortal Wkly Rep **1999**; 48:707–10.
4. Daum RS, Ito T, Hiramatsu K, et al. A novel methicillin-resistance cassette in community-acquired methicillin-resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds. J Infect Dis **2002**; 186: 1344–7.
5. Dufour P, Gillet Y, Bes M, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin Infect Dis **2002**; 35:819–24.
6. Kravitz G, Dries DJ, Peterson ML, Schlievert PM. Purpura fulminans due to *Staphylococcus aureus.* Clin Infect Dis **2005**; 40:941–7.
7. Ladhani S. Recent developments in staphylococcal scalded skin syndrome. Clin Microbiol Infect **2001**; 7:301–7.
8. McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. Annu Rev Microbiol **2001**; 55: 77–104.
9. Todd JK, Kapral FA, Fishaut M, Welch TR. Toxic shock syndrome associated with phage group 1 staphylococci. Lancet **1978**; 2:1116–8.
10. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol **2003**; 48:1429–49.
11. Yarwood JM, Schlievert PM. Quorum sensing in *Staphylococcus* infections. J Clin Invest **2003**; 112:1620–5.
12. Pragman AA, Schlievert PM. Virulence regulation in *Staphylococcus aureus:* the need for in vivo analysis of virulence factor regulation. FEMS Immunol Med Microbiol **2004**; 42:147–54.
13. McCormick JK, Bohach GA, Schlievert PM. Pyrogenic, lethal, and emetic properties of superantigens in rabbits and primates. Methods Mol Biol **2003**; 214:245–53.
14. Lina G, Bohach GA, Nair SP, Hiramatsu K, Jouvin-Marche E, Mariuzza R. Standard nomenclature for the superantigens expressed by *Staphylococcus.* J Infect Dis **2004**; 189:2334–6.
15. Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. Science **1990**; 248:705–11.
16. Kotzin BL, Leung DY, Kappler J, Marrack P. Superantigens and their potential role in human disease. Adv Immunol **1993**; 54:99–166.
17. Choi Y, Lafferty JA, Clements JR, et al. Selective expansion of T cells expressing V beta 2 in toxic shock syndrome. J Exp Med **1990**; 172: 981–4.
18. Boguniewicz M, Schmid-Grendelmeier P, Leung DY. Atopic dermatitis. J Allergy Clin Immunol **2006**; 118:40–3.
19. Homey B, Steinhoff M, Ruzicka T, Leung DY. Cytokines and chemokines orchestrate atopic skin inflammation. J Allergy Clin Immunol **2006**; 118:178–89.
20. McGirt LY, Beck LA. Innate immune defects in atopic dermatitis. J Allergy Clin Immunol **2006**; 118:202–8.
21. Hauk PJ, Hamid QA, Chrousos GP, Leung DY. Induction of corticosteroid insensitivity in human PBMCs by microbial superantigens. J Allergy Clin Immunol **2000**; 105:782–7.
22. Schlievert PM, Case LC, Strandberg KL, Tripp TJ, Lin YC, Peterson ML. Vaginal *Staphylococcus aureus* superantigen profile shift from 1980 and 1981 to 2003, 2004, and 2005. J Clin Microbiol **2007**; 45:2704–7.
23. Schlievert PM. Immunochemical assays for toxic shock syndrome toxin-1. Methods Enzymol **1988**; 165:339–44.
24. Blake MS, Johnston KH, Russell-Jones GJ, Gotschlich EC. A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal Biochem **1984**; 136:175–9.
25. ImageJ: image processing and analysis in java. Available at: http://rsb.info.nih.gov/ij/. Accessed 17 March 2008.
26. Pragman AA, Yarwood JM, Tripp TJ, Schlievert PM. Characterization of virulence factor regulation by SrrAB, a two-component system in *Staphylococcus aureus.* J Bacteriol **2004**; 186:2430–8.
27. Yarwood JM, McCormick JK, Schlievert PM. Identification of a novel two-component regulatory system that acts in global regulation of virulence factors of *Staphylococcus aureus.* J Bacteriol **2001**; 183: 1113–23.
28. Pragman AA, Herron-Olson L, Case LC, et al. Sequence analysis of the *Staphylococcus aureus srrAB* locus reveals that truncation of *srrA* affects growth and virulence factor expression. J Bacteriol **2007**; 189: 7515–9.
29. Jarraud S, Peyrat MA, Lim A, et al. *egc,* A highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus.* J Immunol **2001**; 166:669–77.
30. De Boer ML, Chow AW. Toxic shock syndrome toxin 1–producing *Staphylococcus aureus* isolates contain the staphylococcal enterotoxin B genetic element but do not express staphylococcal enterotoxin B. J Infect Dis **1994**; 170:818–27.
31. Schlievert PM. Staphylococcal enterotoxin B and toxic-shock syndrome toxin-1 are significantly associated with non-menstrual TSS. Lancet **1986**; 1:1149–50.
32. Schlievert PM, Tripp TJ, Peterson ML. Reemergence of staphylococcal toxic shock syndrome in Minneapolis-St. Paul, Minnesota, during the 2000–2003 surveillance period. J Clin Microbiol **2004**; 42:2875–6.
33. Bohach GA, Kreiswirth BN, Novick RP, Schlievert PM. Analysis of toxic shock syndrome isolates producing staphylococcal enterotoxins B and C1 with use of southern hybridization and immunologic assays. Rev Infect Dis **1989**; 11(Suppl 1):S75–82.
34. Betley MJ, Lofdahl S, Kreiswirth BN, Bergdoll MS, Novick RP. Staphylococcal enterotoxin A gene is associated with a variable genetic element. Proc Natl Acad Sci USA **1984**; 81:5179–83.
35. Musser JM, Schlievert PM, Chow AW, et al. A single clone of *Staphylococcus aureus* causes the majority of cases of toxic shock syndrome. Proc Natl Acad Sci USA **1990**; 87:225–9.
36. Yarwood JM, Schlievert PM. Oxygen and carbon dioxide regulation of toxic shock syndrome toxin 1 production by *Staphylococcus aureus* MN8. J Clin Microbiol **2000**; 38:1797–803.
37. Hill DR, Brunner ME, Schmitz DC, et al. In vivo assessment of human vaginal oxygen and carbon dioxide levels during and post menses. J Appl Physiol **2005**; 99:1582–91.
38. Schlievert PM, Blomster DA. Production of staphylococcal pyrogenic exotoxin type C: influence of physical and chemical factors. J Infect Dis **1983**; 147:236–42.