The staphylococcal enterotoxin (SE) family

SEB and siblings

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Introduction

*Staphylococcus aureus* plays an important role in numerous human cases of food poisoning, soft tissue, and bone infections, as well as potentially lethal toxic shock. This common bacterium synthesizes various virulence factors that include staphylococcal enterotoxins (SEs). These protein toxins bind directly to major histocompatibility complex class II on antigen-presenting cells and specific Vβ regions of T-cell receptors, resulting in potentially life-threatening stimulation of the immune system. Picomolar concentrations of SEs ultimately elicit proinflammatory cytokines that can induce fever, hypotension, multi-organ failure, and lethal shock. Various in vitro and in vivo models have provided important tools for studying the biological effects of, as well as potential vaccines/therapeutics against, the SEs. This review succinctly presents known physical and biological properties of the SEs, including various intervention strategies. In particular, SEB will often be portrayed as per biodefense concerns dating back to the 1960s.

**Staphylococcus aureus** is a formidable pathogen linked to many human diseases.1,3 Planktonic and sessile (biofilm-based) versions of *S. aureus* can occur in an infected host. This facultative, β-hemolytic, gram-positive, halo-tolerant bacterium readily colonizes skin, various mucosal surfaces, soft tissues, and bone, as well as indwelling medical devices. Approximately 30% of humans are asymptomatic carriers of *S. aureus* harboring genes for antibiotic-resistance, staphylococcal enterotoxins (SEs), and other virulence factors.4 Within the non-institutionalized population of the US, Caucasian males less than 65 years old and possessing minimal education are those most likely colonized by *S. aureus*. Another interesting finding by Graham et al. reveals that SEB is strongly correlated with methicillin-resistant strains of *S. aureus* (MRSA).4

In addition to the SEs that stimulate specific subsets of T cells,2,5 *S. aureus* also possesses many other virulence factors that include adhesins, collagenases, protein A, coagulases, hemo-lysins, and leukocidins.2,3,6 Clearly, the bacterium is very adept at surviving in/on a host via a hefty, diverse arsenal. Often mentioned in popular and scientific literature is an ever-increasing resistance of *S. aureus* toward antibiotics like methicillin and now vancomycin, which represents a serious societal concern for both humans and animals.2,4 In hospitals and nursing homes, antibiotic-resistant strains are a particularly deadly bane. Strict adherence to infection control plans is necessary to check inadvertent spread of *S. aureus* among staff and patients. Indeed, *S. aureus* is an important health and economic concern throughout the world.9 From a biodefense perspective spanning decades of research, SEB is considered a Category B select agent by the Centers for Disease Control and Prevention that is harmful following inhalation.10,11

When naturally derived by ingestion, the SEs (A–U, and counting) are associated with one of the most prevalent forms of food poisoning found throughout the world.2,12 It is evident that various populations are naturally exposed to these toxins, as demonstrated by SEB seroconversion rates in humans.13 Whether toxin-specific antibodies are developed after ingesting contaminated food, and/or colonization of humans by a toxin-producing strain of *S. aureus*, is to date unknown. Furthermore, whether pre-existing antibody titers in some individuals among the normal population protect against a biological attack using SEB remains an unanswered question.

SE poisoning naturally occurs after ingesting processed meats or dairy products previously contaminated by improper handling and storage. Such conditions are conducive to *S. aureus* growth, and pending strain, release of one (or more) SEs into the tainted food. Only microgram quantities of consumed toxin are needed to cause emesis and diarrhea within approximately 4 h, and one may still experience a general malaise 24 to 72 h later.14 As food poisoning by SEs is non-fatal and of short duration, supportive care is indicated and includes over-the-counter medication for symptomatic relief of gastrointestinal discomfort. Little effort is devoted toward developing countermeasures of foodborne illness induced by SEs. Poisoning by the SEs via many different food types is rarely fatal for healthy individuals, and occurs around the world; however, the very young and old represent higher risk groups.15 Furthermore, recent murine studies suggest that low, chronic levels of SEB can also experimentally induce autoimmunity.16 This brings up an interesting, yet largely unexplored, aspect of health effects upon humans following chronic colonization by toxin-producing *S. aureus*. We foresee future work in this area of toxin-induced autoimmunity becoming interestingly fruitful.
Exactly how the SEs cause enteric illness is still remarkably unresolved, but prostaglandins and leukotrienes may mediate the effects.3,18 In addition to causing food poisoning, the SEs (historically SEB) have a nefarious potential for biological warfare and bioterrorism.10,11 After inhalation, SEB can induce within 2 h several symptoms that include: head and muscle aches, tachycardia, coughing, nausea, vomiting, diarrhea, and conjunctiva irritation.10 These forms of incapacitation occur at nanogram levels, while microgram quantities of SEB can be fatal. As the toxin alters immunity, it is plausible that other agents (viral and bacterial) may act in a synergistic/opportunistic manner with co-administered SEB. Again, from a biodefense or civilian medical perspective, enhancement of opportunistic infections via co-exposure to bacterial toxin(s) is an unexplored (and admittedly complex) area of research.

Pre-existing antibodies do play an important role in susceptibility to staphylococcal toxic shock elicited by toxic shock syndrome toxin-1 (TSST-1).19 As described below, similar circumstances exist for the SEs in various animal models. Individuals not seroconverted toward TSST-1 due to toxin-induced hypersensitive T cells,20 and/or T-cell-dependent B cell apoptosis,21 are more likely to relapse. Perhaps these findings emphasize a need for vaccines that may break tolerance toward TSST-1, and other staphylococcal superantigens, especially among high-risk populations.22-27

The therapeutic use of immunoglobulins can help prevent staphylococcal-induced shock. Intravenous immunoglobulin (IVIg), pooled from human donors, is particularly beneficial in the clinic but problems exist with batch to batch variation that logically include neutralizing titers and targeted antigens.28,29 Use of humanized monoclonal antibodies, in a “cocktail” targeting unique epitopes on the SEs and TSST-1, represents a logical step forward.30 This is akin to that described for Clostridium botulinum neurotoxin A, another bacterial protein that is of high concern within the biodefense community.31

“Superantigen”, a term used often in this review, commonly describes the SEs, TSST-1 and structurally related streptococcal pyrogenic exotoxins (SPEs) of Streptococcus pyogenes. This designation originated in the late 1980s to define microbial proteins that activate a large population of specific T cells at picogram levels.32-35 Superantigens are in contrast with “conventional” antigens that typically stimulate far fewer T cells at higher concentrations. Superantigen interactions with host cells further differ from conventional antigens by: (1) direct interactions on the outside of the peptide-binding groove of major histocompatibility complex class II (MHC II), (2) binding to various MHC II types, and (3) exerting biological effects upon the host without internalization and antigen processing.32-34 Additionally, recognition of a superantigen-MHC II complex by the T-cell receptor (TCR) depends upon the variable region within a TCR variable β chain (Vβ), and not a Vα-Vβ chain combination commonly used by conventional peptide antigens.34 Microbial superantigens are also produced by other bacteria and even viruses, thus suggesting a conserved and successful strategy employed throughout nature.

**Toxin Structure and Receptor Binding**

The SEs and TSST-1 are 22- to 30-kD, single-chain proteins secreted by *S. aureus* that form distinct homology groups based upon amino acid sequence.2,5 There are more than 20 SE variants described in the literature. Furthermore, there are approximately ten SE-like (SEL) proteins produced by *S. aureus* that lack luminal properties or have not been tested to date.35 Among the different SE “serotypes” originally described decades ago, SEA, SEB, and SEE share the highest amino acid sequence homology ranging from 53% to 81%. SEB is 50–66% homologous with SECs (1, 2, and 3 subtypes).2,5

Despite varying sequences, structural studies, and X-ray crystallography of SEA, SEB, SEC2, and TSST-1 reveal quite conserved conformations with two tightly-packed domains containing β-sheet plus α-helix structures separated by a shallow groove.36,37 Structure-function studies with site-directed mutagenesis and overlapping peptides of these toxins, along with crystallographic analysis of toxin–MHC II complexes, provide further clues regarding specific residues critical for binding to MHC II and TCR.26,28-30 The SEs and TSST-1 additionally share similar structures (i.e., epitopes) as evidenced by cross-reactivity and neutralization with antibodies.22-27,40-42

**Figure 1** shows two orientations of SEB and regions involved in binding to murine TCR (Vβ 8.1) as well as human MHC II (HLA-DR1).37,39,43

The staphylococcal superantigens bind to conserved elements of MHC II with high micro- to low nanomolar affinity.2,5,44,45 However, each toxin preferentially binds to distinct alleles which suggests different contact sites on MHC II. Upon comparing the binding attributes of staphylococcal superantigens, SEA has the highest affinity for HLA-DR1 mediated by two binding sites.

Co-crystals of SEB or TSST-1, complexed with HLA-DR1 and associated peptide antigen, also clearly reveal distinct binding differences between these toxins.33,46 For example, SEB interacts exclusively with the α chain of HLA-DR1 and is unaffected by the associated peptide. Overall, it is clear that diverse methods exist for SEs and TSST-1 binding to both MHC II and TCR, which can partly explain differential activation of T cells.47,48

The groove formed between conserved domains of staphylococcal superantigens represents an important interaction site for the TCR Vβ chain (Fig. 1).38,39,48 Each toxin binds to a distinct repertoire of Vβ-bearing T cells, thus displaying a unique biological “fingerprint” that might be helpful in the clinic for diagnosing superantigen exposure.47 Mutations within the MHC II binding domains of SEA differentially affect binding to TCR Vβ,49 as evidenced by a small increase in superantigen affinity for MHC II, thus overcoming a large decrease in affinity for TCR Vβ. Furthermore, disulfide-linked homodimers of CD28 represent an additional binding site for SEB important for T-cell stimulation and subsequent biological effects.50 This same study interestingly reveals direct binding of SEB to CD28, without MHC II or TCR.
Recognition of the superantigen/MHC II complex by TCR results in cell signaling, proliferation, and subsequent release of cytokines/chemokines. Immune cell activation by superantigens and subsequent cellular changes are similar to those of conventional antigens and requires three important signals. Figure 2 shows the cells and mediators involved in eliciting the biological effects of superantigens. Signal 1 comes from superantigen interaction with TCR and activation of protein tyrosine kinases (PTKs), which in turn phosphorylate tyrosine-based motifs of the TCR intracellular components and other cellular substrates plus adaptors. Activation of phospholipase C gamma (PLCγ) through phosphorylation by TCR-induced kinases generates second messengers. The latter subsequently activate protein kinase C (PKC), the proto-oncogene Ras, and also increase intracellular calcium levels. Engagement of costimulatory molecules on antigen-presenting cells (APCs) and T cells, upon superantigen binding, results in a second signal that optimizes T-cell activation. Expression of intercellular adhesion molecule (ICAM) on an APC promotes stable cell conjugates and an immunological synapse. The interactions between adhesion (LFA-1 with ICAM-1) and costimulatory (CD28 with CD80) molecules have been implicated in superantigen-mediated T-cell responses.

![Figure 1. Crystal structure of SEB at 1.5 Å from Papageorgiou et al. using the Molecular Modeling Database (MMDB) of the National Center for Biotechnology Information (NCBI). Two different orientations reveal SEB residues important for binding to (A) murine TCR (Vβ8.1) and (B) human MHC II (HLA-DR1).](image-url)
activation. Other cell-surface molecules such as CD2, CD11a/ICAM-1, and ELAM facilitate optimal activation of endothelial cells and T cells by SEB. PKC and PTK activation lead to other downstream signaling pathways including mitogen-activated protein kinase (MAPK), extracellular signal regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) pathways, ultimately activating transcriptional factors NFkB, NF-AT, and AP-1. Many proinflammatory cytokine genes contain NFkB binding sites within the promoter/enhancer region and are induced by NFkB. Interleukin (IL)-1, tumor necrosis factor (TNFα), interferon gamma (IFNγ), IL-2, IL-6, and chemokines, specifically monocyte chemotactant protein-1 (MCP-1), are induced directly by superantigens and represent the third signal for T-cell activation. IL-1 and TNFα can also activate fibroblasts plus endothelial and endothelial cells to produce other mediators, thus providing an inflammatory environment for T-cell activation. The binding of TCR, costimulatory receptors, T-cell cytokines (IL-2 and IFNγ) plus chemokines to their respective receptors activate the lipid kinase, phosphoinositide 3 kinase (PI3K), which in turn activates the mammalian target of rapamycin (mTOR). The PI3K/Akt/mTOR pathway regulates many physiological and pathological processes as it controls cell survival, growth and migration. Myeloid differentiation protein 88 (MyD88) is also an adaptor protein involved in SEB-induced cytokine signaling. Clearly, SEB-based activation of cells involves a multi-factorial event encompassing multiple host molecules. Biological effects induced by superantigens are triggered by low, non-saturating occupancy rates indicative of “low affinity” binding to MHC II.

Human whole blood and purified peripheral blood mononuclear cells (PBMCs) are commonly used in vitro to study cell activation by staphylococcal superantigens, as well as potential therapeutic agents against these toxins. PBMCs release cytokines and chemokines following SE or TSST-1 exposure, such as: IL-1, IL-2, IL-6, TNFα, IFNγ, macrophage inflammatory protein 1α (MIP-1α), MIP-1β, and MCP-1. Although monocytes alone can produce many chemokines as well as proinflammatory cytokines, like IL-1, IL-6, and TNFα, T cells enhance mediator levels. There are contradictory reports regarding APCs and T-cell responses to these bacterial toxins without the other cell type, as evidenced by cytokine/chemokine production by human monocytic lines or freshly isolated cells. MHC II-linked stimulation of T cells by SEs is a general requirement, but those with select TCR Vβs can independently respond with less efficiency.

Additional cell types that respond to superantigens include B, nasal, intestinal and vaginal epithelium, as well as intestinal fibroblasts and synovial myofibroblasts. The cross-linking of TCR with MHC II by superantigen triggers B-cell proliferation and differentiation into immunoglobulin-producers in a dose-dependent manner, but high concentrations of superantigen inhibit immunoglobulin synthesis. Suppression of antibody synthesis by TSST-1 reportedly occurs via apoptosis, which can clearly hamper protective immunity against this toxin. Such an effect upon B cells is likely linked to recurring bouts of toxic shock following persistent, vaginal colonization by TSST-1 producing S. aureus. Upon activation by SEB, nasal epithelial cells produce granulocyte colony-stimulating factor and various chemokines including MCP-1. Transcytosis of SE across intestinal epithelial cells has been observed in vitro, and in vivo the toxin penetrates the gut lining that then leads to local and systemic immune responses.

In Vivo Effects: Animal Models and More

Monkeys: the laboratory standard (but expensive!)

The SEs readily induce emesis in primates when ingested, but not after i.v. injection, in low microgram quantities. P By the dose plus route, there may be more severe consequences that progress from rapid hypotension and decreased cardiac output, into lethal toxic shock not reversed by epinephrine. Humans are more sensitive to ingested SEB than monkeys. For many years, classic primate studies for SEs have been performed by various groups and are the “gold standard”. However, this animal model has become prohibitively expensive in many ways that obviously includes money, but also institutional relations with the lay public. In contrast to the SEs, structurally related TSST-1 and some tested SEL proteins do not cause vomiting in monkeys after ingestion.

Unlike many other bacterial enterotoxins, specific cells and receptors in the intestinal tract have not been clearly associated with SE intoxication. The latter seemingly requires a complex interplay between immunological and non-immunological mechanisms involving multiple cell types. SEB stimulation of mast cells causes release of cysteinyl leukotrienes that subsequently
Studies with human Caco-2 monolayers reveal transcytosis of SEA, SEB, and TSST-1, with ingested SEB entering the bloodstream of mice more readily than SEA. These data suggest that SEs cross the gastric mucosa and circulate throughout the body. In vitro, the SEs are not cytotoxins that directly disrupt human (Henle 407) intestinal cells. However, SEB affects the gut mucosa as evidenced by increased ion flow through a monolayer of human T84 colonic cells incubated with SEB-stimulated PBMCs. The interactions of most superantigens with epithelial cells are indirect via release of IL-1, TNF-α, and IFN-γ from super-antigen-activated APCs and T cells. Furthermore, there is an age-related increase (up to ten years among humans) in CD4+ T cells that produce IFN-γ following SEB exposure, thus increased susceptibility to SEs over time is linked to IFN-γ levels.

Although debatable, it appears that superantigenicity may not play a role in SE enterotoxicity as recombinant variants of SEA and SEB lacking MHC II binding and T-cell mitogenicity remain emetic. Carboxymethylation or tyrosine replacement of SEA and SEB lacking MHC II binding and T-cell mitogenicity does not play a role in SE enterotoxicity as recombinant variants of SEB lacking MHC II or H187 do not alter wild-type properties. As stated before, affinity for MHC II and specific TCR VB enables superantigens to induce high levels of proinflammatory cytokines. The SEs and TSST-1 are pyrogenic in primates as well as rabbits, a likely result of elevated levels of proinflammatory cytokines that include IFN-γ, IL-1, and TNF-α. IFN-γ enhances immunological responses by elevating expression of MHC II on APCs and epithelial/endothelial cells, as well as augments the proinflammatory actions of IL-1 and TNF-α. SEB causes acute lung injury and potential shock characterized by increased: (1) expression of adhesion molecules like ICAM-1 and vascular cell adhesion molecule (VCAM), (2) neutrophil and mononuclear cell infiltration, (3) endothelial cell injury, (4) serum levels of proinflammatory cytokines, and (5) vascular permeability.

Mice: experimentally not perfect, but often preferred

Mice have historically been used by various groups as an alternative to monkeys for studying superantigen-mediated effects in vivo. From a cost perspective, mice are very feasible for basic toxin studies and discovery of therapeutics/vaccines for combating staphylococcal superantigen-induced shock. However, mice lack an emetic response and are thus questionably appropriate for studying the food poisoning aspects of SEs. Nasal application of SEB in mice elicits IL-1, IL-2, IL-6, IFN-γ and lung injury still evident four days after steroid treatment. Interestingly, IL-17 plays a critical role in allergic rhinitis as evidenced by experiments with knockout mice not responding to SEB. Nasal and systemic effects toward an SEB vaccine applied to the nares, or perhaps even mucosa-dwelling S. aureus, are linked to nasopharynx-associated lymphoid tissue. When given intrarectally to mice, SEA or SEB elicit an intestinal inflammatory response and exacerbate a preexisting, microbial-based syndrome (inflammatory bowel disease (IBD) that further suggests an immunological-based component provided by the host.

### Table 1. Enteric, lung, and skin models for staphylococcal superantigens

| Animal | Inducing agent(s) | Route | Mediators, symptoms, pathology |
|--------|------------------|-------|-------------------------------|
| Mouse Balb/c | SEB | i.g. | IFN-γ and IL-2 increase in mucosal lymphoid tissue at 4 h |
| Mouse HLA-DR3 IFN-γ knockout | SEB | i.p. | IFN-γ linked to intestinal pathology, increased gut permeability, toxic shock |
| Mouse C57BL/6 | SEB | i.p. | Acute lung inflammation, leukocyte infiltration, capillary leakage, and endothelial cell injury by 6 h |
| Mouse (different strains) with inflammatory bowel disease | SEA or SEB | i.r. | Exacerbation of inflammatory bowel disease |
| Monkey cynomolagus | SEB | i.d. | Immediate-type skin reaction, emesis, cutaneous mast cell degranulation, and cysteinyl leukotriene generation |
| Monkey cynomolagus | SEA, SEB, or SEC | i.g. or i.v. | Emesis at 3 h, followed by diarrhea |

i.d., intradermal; i.g., intragastric; i.p., intraperitoneal; i.r., intrarectal; i.v., intravenous.
superantigenicity, become resistant to a subsequent lethal challenge of SEA but not TSST-1. This form of tolerance is not linked to toxin-specific antibody or deletion/anergy of SEA-reactive T cells. However, a significant increase in serum IL-10 levels among these animals correlates with in vitro and in vivo protection against SE-induced effects. IL-10, but not IL-4, provides protection against abnormal ion flow through a human colonic monolayer following SEB stimulation of PBMCs.

Many of our mouse studies with SEs and TSST-1 have been done via LPS-potentiation with a lethal endpoint, as it has been well established by many laboratories that a natural synergy exists between these protein toxins and LPS. SEs and TSST-1 synergize with LPS many log-fold, minute quantities of each can cause severe effects in mammals. Among healthy humans there are numerous gram-negative bacteria constituting normal intestinal, or vaginal, flora. Along with a recognized increase in these microbes among toxic shock patients, the odds of this superantigen-LPS synergy naturally occurring are plausibly high. There is a strong correlation between elevated serum levels of various proinflammatory cytokines (IL-1, IL-2, TNFα, and/or IFNγ) with SE- or TSST-1-induced shock.

The interdependent effects of SEB used alone, and together with LPS, on serum cytokines/chemokines has been described in further detail using a Balb/c mouse model. SEB alone induces moderate serum levels of IL-2 and MCP-1, with all mice surviving a high dose (100 μg/animal). Additionally there are only low levels of TNFα, IL-1, IFNγ, and MIP-2; however, with LPS there is increased expression of these cytokines to include IL-6 and MCP-1. Thus, the synergistic action of SEB and LPS promotes early TNFα release and prolongs IL-6, IFNγ, IL-2, MIP-2, and MCP-1 release in non-survivors. Overall, the elevated and sustained levels of these key cytokines lead to lethal toxic shock within 48 h after co-administration of LPS plus SEB. Mice given an antibody against IL-10 have increased serum levels of IL-2, IFNγ, plus TNFα after SEB stimulation, and become more susceptible to lethal shock. Additionally, these efforts correlate nicely with others employing SEA and knockout mice lacking IFNγ, IL-2, or TNF receptor type 1. Further work has been described with SE or TSST-1 intoxication among knockout deficiencies in IL-10, TNF receptor types I or II, or CD43.

Besides knockouts, another method for studying superantigenic shock in mice includes transgenics with inserted genes, often of human origins. As one example, mice expressing human HLA-DQ6 and CD4 succumb to normally sublethal amounts of SEB (with β-galactosamine potentiation), and the serum levels of TNFα correlate with lethal shock. In particular, two high doses of SEB (30 and 100 μg/mouse) are necessary to induce toxic shock in this model. Regarding mode of action, β-galactosamine is converted by hepatocytes into uridine diphosphate-galactosamine, which in turn prevents uridine triphosphate formation. Ultimately this affects RNA, and subsequent protein, synthesis that becomes lethal for the host.

Transgenic mice with human HLA-DR3 lethally respond to SEs without a potentiating agent, thus providing a “simpler” model for future in vivo toxin studies. Like the HLA-DR3 model, transgenic (HLA-DQ8) mice also respond to SEB without a potentiating agent as per elevated serum levels of various cytokines. Following SEB exposure (aerosol) in these latter transgenics, the lungs, lesions, temperature fluctuations, and lethality after 96 h are also similar to those experienced by monkeys after a lethal aerosol.

Besides mice displaying human receptor on cells, transgenics that overexpress murine TCR Vβ3 also have increased mortality linked to elevated TNF and IFNγ levels following infection by SEA-producing S. aureus. Overall, transgenic animals can provide interesting clues to superantigenic shock in humans at a fraction of the cost vs. monkeys.

In addition to lethality as an endpoint, temperature has been used for studying SE and TSST-1-induced shock in mice. These studies were accomplished by implanting a subcutaneous transponder or intraperitoneal telemetry device, in which the latter also measured movement. Telemetry technology affords a seamless collection of data without human handling, thus negating some potential confounding factors during an experiment. There is a rapid (within 10 h) temperature decrease readily evident post-toxin injection of mice, thus providing a rapid non-lethal model. Temperature, but not movement, significantly correlates with SEB intoxication. None of these studies detected a temperature increase, evident in monkeys, thus suggesting a very rapid onset of shock in these murine models. Intranasal administration of SEB has also been used in various murine models. One consists of a two-hit (or dual-dosing) model in C3H/HeJ mice requiring SEB given two hours apart. The first dose is delivered i.n. and the next administered either i.n. or i.p. Increased serum levels of IL-2, IL-6, and MCP-1, accompanied by elevated lung levels of MCP-1, are evident in this dual-dosing model. MCP-1, a potent activator and chemotactic factor for T cells plus monocytes probably contributes to early leukocyte recruitment into the lung. Pathological lesions, temperature fluctuations, and time course of lethality following SEB exposure also resemble those in transgenic mice and monkeys. In summary, a few mouse (and other animal species) models for the staphylococcal superantigens are shown in Table 2.

The rapid, SEB-induced hyperactivation and proliferation of select Vβ T cells in mice eliminates most of these T cells within 48 h by activation-induced cell death via faulty activating protein-1 (AP-1) transcription factor. After SEB injection of mice, splenic Vβ8 T cells are physically deleted or non-responsive (anergic) to homologous toxin and produce less IL-2 and IFNγ. Others report that these anergic cells can secrete more IFNγ that mediates toxic shock after a subsequent dose of SEB. An evident paradox is that an anti-inflammatory cytokine like IL-10, which protects against SE-induced shock, is also produced by SEB-primed T cells. This perhaps is the host’s attempt to counter the proinflammatory effects of IFNγ? It is possible that SEB-induced anergy differentially affects CD4+ and CD8+ T cells, with the former becoming more susceptible.

Rabbits: a common model for TSST-1

In addition to mice, rabbits have also afforded a reliable in vivo model for SE, and particularly TSST-1, induced shock as determined by temperature and lethal endpoints (Table 2).
Some of these models employ an implanted infusion pump that delivers toxin over time, thus mimicking more naturally a S. aureus infection that leads to toxic shock.112 As evidenced in mice with the various staphylococcal superantigens, different rabbit strains also possess varying susceptibility toward TSST-1 as New Zealand whites are more susceptible vs. Dutch belted.113 As witnessed in humans with toxic shock, rabbits given TSST-1 or SEB experience elevated levels of circulating LPS eliminated by polymyxin B.113,114 From a biodefense perspective, intrabronchial instillation of SEB (SEC or TSST-1 too) into rabbits can be useful for testing potential therapies and vaccines.115 The testing of live S. aureus is also possible in this model for exploring interventions against bacterial pneumonia.

**Goats, ferrets, and shrews: unusual alternatives**

In addition to monkeys, mice, and rabbits, other less employed models for SE intoxication have been described in the literature. For example, goats have been used for studying in vivo effects of TSST-1 and SEB (0.02–20 μg/kg) after i.v. administration.116 Following SEB exposure, goats experience tachycardia and diarrhea, as well as elevated blood urea plus temperature. In contrast, TSST-1 does not elicit diarrhea in goats, which mimics results from monkeys given this same toxin orally.2,14,35

There is also a ferret model for oral SEB and SEC2 intoxication which elicits emesis, increased defecation, altered feces appearance, and rapid fever; however, this model employs milligram quantities of toxin (upwards to 10 mg/animal) that are much higher than that typically used in various mouse, monkey, or goat models. Possible explanations for requiring this large dose of SEB include receptor differences and/or more efficient degradation of SEB in the gut of ferrets vs. humans or monkeys.

Finally, another emetic model more recently described and highly developed for SE studies employs an unusual laboratory animal: the house musk shrew.118,119 When given orally, both SEA and SEC cause emesis (100 or 500 μg/animal) within 30 to 120 min.119 SEA is more effective than SEC, while TSST-1 has no effect. This model has been useful for vaccine efforts against SEA, using a recombinantly-attenuated SEA devoid of superantigen and emetic activity.118 Furthermore, sera from these vaccinated animals (vs. alum only controls) inhibit SEA (wild type)-induced proliferation of naïve shrew splenocytes (in vitro) as well as emesis. Interestingly, there are no diarrheic effects in shrews when toxin is given orally or injected directly into intestinal loops. Upon comparing models a much lower amount of SE is required in shrews, vs. ferrets, via an i.p. or oral route. However, an unpleasant readout with any emetic (or diarrheic) model is an unpleasant readout with any emetic (or diarrheic) model is

| Table 2. Toxic shock models for staphylococcal superantigens |
|---------------------------------------------------------------|
| **Animal** | **Inducing agent(s)** | **Route** | **Mediators, symptoms, pathology** |
|------------|------------------------|---------|---------------------------------|
| Mouse Balb/c | TSST-1 + LPS | i.v. | TNFα peaks at 1 to 2 h, lethal shock* |
| Mouse Balb/c | SEB + LPS | i.p. | TNFα peaks at 1 h, IFNγ, IL-1, IL-6 increase at 2 h, lethal shock, and hypothermia* |
| Mouse Balb/c | α-galactosamine + SEB | i.p. | High levels of TNFα and IL-2 leading to lethal shock* |
| Mouse Balb/c | Actinomycin D + SEB | i.p. | Blood congestion in lungs and intestine by 4 h, PBMCs in lungs, spleen, and liver, alveolar septa thickening at 8 h, lethal shock at 2 to 4 d* |
| Mouse C3H/HEJ | SEB + SEB | i.n. | Bronchiolar epithelial degeneration, lung neutrophilic infiltration, IL-2, IL-6, and MCP-1 in serum and lung, lethal shock at 96 h* |
| Mouse transgenic HLA-DR3 | SEB | i.n. | Neutrophilic infiltration, TNFα, IFNγ, IL-6, IL-12, and MCP-1 increase at 3 h* |
| Rat Sprague−Dawley | Catheterized, SEB + LPS | i.v. | TNFα increase at 90 min, IFNγ at 4 h, hepatic injury and dysfunction* |
| Rabbit Dutch Belted | TSST-1 + LPS | i.v. | TNFα peaks at 4 h leading to lethal shock* |
| Rabbit Dutch Belted | SEC + LPS | i.v. | Fever at 4 h, hypothermia, labored breathing, diarrhea, vascular collapse, lethality by 24 h* |
| Rabbit New Zealand white | SEA | i.v. | TNFα, IFNγ, and IL-2 increase at 1 to 2 h, peak at 3 to 5 h, febrile reaction evident at 1 h* |
| Monkey Rhesus | SEB | aerosol | Leukocyte infiltration, intra-alveolar edema, parenchymal cell degeneration, lymphocyte necrosis, temperature fluctuation, and lethal shock* |

i.n., intranasal; i.p., intraperitoneal; i.v., intravenous.

The testing of live S. aureus is also possible in this model for exploring interventions against bacterial pneumonia.
and TSST-1 are not lacking for available animal models that can answer, with limitations, some very important questions linked to human intoxication.

Neutralization Strategies

Neither small-molecular weight therapeutics nor vaccines against SEs or TSST-1 have been approved for human use by the United States Food and Drug Administration (FDA). Given that the medical community has fewer effective tools to thwart evolving strains of \textit{S. aureus}, as well as other antibiotic-resistant pathogens, progress toward discovering and subsequently developing therapeutics plus vaccines seem logical. The biodefense concern of defending against a toxin, such as SEB, is a relatively simpler “static” scenario (i.e., toxin does not reproduce) vs. overcoming a bacterial pathogen employing various virulence factors. Mitigation of SE or TSST-1 toxicity will afford some relief for an infected patient suffering from an antibiotic-resistant strain of \textit{S. aureus}. Upon current understanding of SE and TSST-1 intoxication at a molecular level, potential therapies/vaccines should target at least one of three important steps: (1) TCR–toxin–MHC II interactions, (2) accessory, co-stimulatory, or adhesion molecules that include intracellular signaling molecule and adaptor (i.e., CD28, MyD88, etc.) activation of T cells, and (3) cytokine release by activated T cells and APCs. There are clearly multiple targets awaiting further investigation. The question becomes one of funding a focused endeavor(s) that leads to novel findings and a product of clinical value.

Non-immunoglobulin based protection

Attempts at in vitro and in vivo inhibition of the above toxin-exploited targets have been many and diverse, emerging from groups throughout the world. A conserved region (residues 150–161) from SEB prevents shock induced by SEB, as well as SEA or TSST-1, in mice when given 30 min after toxin. This peptide evidently stops transcytosis of various SEs and TSST-1 across a human colonic cell (T84) monolayer, and may block co-stimulatory signaling necessary for T-cell activation. However, subsequent studies indicate that such peptides are ineffective inhibitors of SEB-induced effects both in vitro and in vivo. Another study with a different SEB peptide (residues 72–86) reveals inhibition of SEA-, SEB-, and SEC-mediated responses in vivo. Recently, short synthetic peptides corresponding to the binding region of CD28 were shown to block SEB-induced TNFα, IFNγ, and IL-2 expression. Clearly this is an exciting area of research, but varied results from various groups are troublesome and suggest more work to ascertain any potential usefulness in the clinic. Furthermore, one potential problem for medicinal use of any peptides or proteins involves proteolysis by the host, and thus inactivation.

A different approach for blocking receptor interactions of SEB uses a chimera of the DRα1 domain from MHC II and TCR Vβ connected by a flexible linker. This construct prevents cellular activation and subsequent IL-2 release in SEB-stimulated PBMCs (human) in vitro. A potential drawback is that individual chimeras must be constructed for each SE, as TCR Vβ preferences differ among superantigens. Related to receptor blocking, another group reports that a soluble TCR Vβ mutant can neutralize SEB, and related SPEA, with picomolar affinity.

Aptamers, consisting of peptide or single-stranded nucleic acid, are a relatively new method to detect or neutralize targets that include protein toxins. Such DNA-based molecules, fished from recombinant libraries, can directly bind SEB and prevent receptor interaction. In particular, aptamer technology could be useful in the food safety industry and detection of various SEs in tainted foods.

Once a staphylococcal superantigen engages surface receptor, blockade of signal pathways within a targeted cell represents the next medicinal option. The complexity of any medical intervention at this stage increases dramatically, as (1) entry of any therapeutic into a cell and (2) subsequent short-circuiting of toxin-induced toxicity represents a very daunting task. Naturally, most cells (APCs being an exception) are rather discriminating toward bringing compounds in from the external milieu. This is a very different mode of intervention, vs. those described above for disrupting toxin-receptor interactions, as signal transduction events are post-exposure and will likely work for various SEs and TSST-1.

Nuclear factor κB (NFκB) is an attractive intracellular target for therapy, as its activation is linked to transcription of many mediators involved in inflammation and carcinoma survival. NFκB precursor is proteolytically processed into a mature form that subsequently governs apoptosis and cell proliferation/migration. Activation of NFκB is influenced by various stimuli, such as bacteria and viruses, involving ubiquitination plus proteolysis of sequestration proteins (known as IκB or inhibitors of kappa beta) in the cytosol. In vitro and in vivo studies have shown that many of the inflammation-associated genes implicated in superantigen-induced lethal shock contain NFκB binding sites in the promoter/enhancer regions. A cyclic, cell-penetrating peptide (29 amino acids designated as cSN50) targeting NFκB nuclear transport attenuates SEB-induced T-cell responses and diminishes inflammatory cytokine levels in mice. There is also reduced liver apoptosis, hemorrhagic necrosis, lung damage and mortality following pre- or post-toxin use of cSN50. When cSN50 (i.p.) is given 30 min before SEB (i.n.), there are reduced levels of pro-inflammatory cytokines and chemokines in the bronchoalveolar space of mice. This compound also attenuates neutrophil/monocyte infiltration into the lung and vascular injury.

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mice from SEB-induced shock in the two-hit SEB-only as well as SEB + LPS models.\textsuperscript{79,130} Dexamethasone at a 1.25–5 mg/kg dose attenuates the hypothermic response to SEB in both toxic shock models, markedly improving survival of mice when administered two to three hours after SEB. Resveratrol, a plant-derived phytoalexin, also acts as an anti-inflammatory that affects cyclooxygenases and NFκB pathways after SEB-induced lung injury in mice.\textsuperscript{131}

Studies with human PBMCs in vitro and a mouse model show that either pentoxifylline or pirfenidone lower proinflammatory cytokine expression, thus abrogating the ill effects of SEB or TSST-1.\textsuperscript{56,57} Pentoxifylline is a FDA-approved xanthine derivative commonly used to improve peripheral blood flow that acts as a phosphodiesterase inhibitor targeting TNF. Pirfenidone is an anti-fibrotic pyridone approved for use in various countries, as a phosphodiesterase inhibitor targeting TNF. Pirfenidone is known as Abatacept or Orencia, is normally indicated for treating rheumatoid arthritis. For biodefense purposes, use of drugs previously approved by the FDA for other indications makes sense. In theory, there should be relatively rapid discovery/approval vs. attempting to discover and subsequently develop/approve novel molecules as unique, effective therapeutics. Various avenues of current biodefense research in the United States explore off-label use of existing FDA-approved drugs.

Another FDA-approved immunosuppressant (rapamycin) protects against SEB-induced shock in mice, even when administered 24 h after SEB.\textsuperscript{139} Ironically, this drug was originally tested as an antifungal agent that ultimately failed for this purpose. Rapamycin (a macrocyclic lactone produced by Streptomyces hygroscopicus) prevents proinflammatory cytokine release and T-cell proliferation, by stopping G1 to S cycling of cells not restricted to T lymphocytes.\textsuperscript{140} Mechanistically, rapamycin binds to an immunophilin (FK-506 binding protein 12 [FKBP12]) acting as a peptidyl-prolyl isomerase important in protein folding and trafficking. The rapamycin-FKBP12 complex blocks mTOR complex 1 activity and inhibits cell cycle progression. Rapamycin represents an effective treatment post-SEB exposure since both TCR and costimulatory molecules, as well as T-cell cytokines, activate the PI3K/Akt/mTOR pathway.

A fruitful target for mitigating proinflammatory cytokine release after LPS or SE/TSST-1 exposure, in vitro and in vivo, is MyD88.\textsuperscript{53,141} Therapeutic inhibition of MyD88-based signaling

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| Pharmacologic agent | Target | Biological effects against SEB |
|---------------------|--------|-------------------------------|
| Mimetic peptides of CD28 dimer interface | Costimulatory molecule CD28 | Attenuated SEB-induced TNFα, IL-2, IFNγ in human PBMC. Protected mice from lethal challenge with SEB by 70%.\textsuperscript{54} |
| Mimetic peptides of BB loop of MyD88 | Toll/IL-1 receptor domain of MyD88 | Reduced SEB-induced IL-1β, IL-1, TNFα and IFNγ in human PBMC. Afforded 83% protection in mouse model of SEB plus LPS-induced shock.\textsuperscript{53,141} |
| Rapamycin (FDA-approved for prevention of renal graft rejection) | Immunophilin FKS068P12 | Blocked SEB-induced MCP-1 and IL-6 in vitro and in vivo. Protected mice 100% from lethality when administered 24 h after SEB.\textsuperscript{139} |
| Dexamethasone (FDA-approved for treating inflammatory diseases) | NFκB | Inhibited SEB-induced proinflammatory cytokines and chemokines in PBMC.\textsuperscript{134} Reduced serum levels of cytokines, attenuated hypothermia due to SEB, and prevented both SEB and SEB + LPS-induced lethal shock in mice.\textsuperscript{79,130} |
| Pentoxifylline (FDA-approved for treating peripheral arterial disease) | Phosphodiesterase | Attenuated SEB-induced proinflammatory cytokines and chemokines in PBMC.\textsuperscript{14,16} Blocked cytokine release in vivo and prevented lethality in SEB + LPS-induced shock model.\textsuperscript{56} |
| Pirfenidone | TGFβ1 | Inhibited SEB-stimulated cytokines in vitro and in vivo. Improved survival of mice against SEB + LPS.\textsuperscript{57} |

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Table 3. Effective small molecule therapeutics for murine models of SEB-induced shock
occurs via binding of a synthetic BB-loop mimic of the Toll/IL-1 receptor domain found on MyD88. Application of such a therapeutic for both endo- or exo-toxic shock is rather appealing from a clinical perspective, apparently possessing universal application. A summary of effective small molecule therapeutics in various murine models of SEB-induced shock is shown in Table 3.

A natural feedback inhibitor of various signal transducers and activators of T cells (STATs) used by IFNγ and IL-2 signaling is the suppressor of cytokine signaling 3 (SOCS3). In this regard, a cell-penetrating form of SOCS3 protects animals from lethal effects of SEB and LPS by reducing inflammatory cytokine production, as well as attenuating liver apoptosis and hemorrhagic effects of SEB and LPS by reducing inflammatory cytokine production. In this regard, activators of T cells (STATs) used by IFNγ not only biodefense, but also civilian, medical concerns involving S. aureus staphylococcal superantigens.

CD44 involved in so many inflammatory processes is logical for controlling this life-threatening disease. In fact, low antibody titers, relative avidities, neutralizing capabilities, amounts of each antibody, etc. One way to minimize batch-to-batch variability of sera of those naturally hyperimmune to various S. aureus toxins involves immunoglobulins, a successful neutralization strategy for over a hundred years against various bacterial toxins. Sometimes the old methods (or modern variants of!) prove to be best, even in light of rapidly evolving technologies evident throughout the biological sciences. It is known that recurring bouts of TSST-1 can be linked to low antibody titers, thus emphasizing the importance of host immune responses in controlling this life-threatening disease. In fact, low antibody titers to various S. aureus exotoxins (i.e., α and δ hemolysins, Panton–Valentin leukocidin, and SEC1) can also portend susceptibility to S. aureus sepsis in hospital patients. Furthermore, IVIg can be effective against staphylococcal-induced shock. These antibody preparations are derived from pooled human sera of those naturally hyperimmune to various S. aureus antigens. As S. aureus readily colonizes humans and grows (when given opportunity) in various consumed foods, seroconversion opportunities against various virulence factors (i.e., SEs and TSST-1) are many. With IVIg, there will naturally be many variables between lots (i.e., antibody recognition of different antigens, relative avidities, neutralizing capabilities, amounts of each antibody, etc). One way to minimize batch-to-batch variability of polyclonal antibodies is to develop human monoclonal antibodies characterized by various assays. Recent studies by different groups target select SEs, including SEB, with different types of antibodies (i.e., recombinant human or mouse–human chimeras) for various purposes (Table 4).

A further twist for deriving characterized immunoreagents for therapy or diagnostic purposes includes recombinant single-domain, heavy-chain antibodies of lama origins that target SEB. Lama antibodies consist of only heavy chains and are

### Table 4. SEB-targeting monoclonal antibodies (mAbs) in recent literature (2010–12)

| Antibody | Species | Specificity | Binding affinity and use |
|----------|---------|-------------|--------------------------|
| Ch63 and Ch82M full-length mAbs | Human–mouse chimeras | Distinct, undefined epitopes on SEB. No cross-reactivity with SEA or TSST-1. | Ch63 = 437 pM (K<sub>d</sub>) for SEB by surface plasmon resonance (SPR). Ch82M = 602 pM (K<sub>d</sub>) for SEB. In vitro neutralization of SEB-induced proliferation in murine splenocytes from BALB/C or HLA-DR3 transgensics. Neutralization of SEB in human PBMC assays. Synergistic protection afforded by mAbs. |
| Ten Fabs with two converted to full-length mAbs | Human | No reactivity with SEA, TSST-1, or SPEA. Varying reactivities with SEB, SEC1, SEC2, and SPEC. | K<sub>v</sub> range of 1.1 μM–1.3 nM for SEB by SPR. In vitro neutralization of SEB, SEC1, and SPEC in human PBMC assays. Neutralization of SEB in lethal shock model (murine). |
| Fabs and full-length mAbs | Human | Varying reactivities with SEA, SEB, SEC1, and SED | K<sub>v</sub> range of three best clones (1.2 nM–320 pM) for SEB by SPR. In vitro neutralization of SEB in human PBMC assays. Neutralization of SEB in lethal shock model (murine). Antibodies are stable toward heat and cold over 15 d. |
| Single domain antibody (sdAb) A3 consisting of only heavy chain | Lama | Specific for SEB, no cross-reactivity with SEA, SED, or Shiga toxin | K<sub>v</sub> range of 75-600 pM for SEB by SPR. Used as capture and detector antibody in Luminox-based assays for SEB (64 pg/ml detection limits). sdAb A3 is heat stable up to 80°C. |
| Fab and single-chain variable fragments (scFv) from commercial mAb (ab53981) | Mouse | SEB only tested with Fab and scFv fragments. Intact ab53981 recognizes N-terminal epitope PDELHKS, as well as SEC2 and SED. | Fab fragment = 4.1 pM (K<sub>d</sub>) for SEB by SPR. Antibody fragments used in establishing detection limits for SEB by ELISA (0.5 ng) and western blot (25 ng). |

**References**
1. CD44 is involved in multiple cell functions, acting as an intracellular signaling protein and receptor for various ligands. CD44 is increasingly expressed among PBMCs in the lung after SEB exposure. Antibody against CD44, or use of CD44-knockouts, mitigates the lung-injuring effects of SEB (i.n.) in mice that include: (1) enhanced vascular permeability, (2) increased cell infiltration, and (3) elevated cytokine levels. Perhaps, like MyD88, targeting of a protein(s) such as CD44 involved in so many inflammatory processes is logical for not only biodefense, but also civilian, medical concerns involving staphylococcal superantigens.

**Immunoglobulin-based protection**

Perhaps one of the more promising therapeutics against S. aureus toxins involves immunoglobulins, a successful neutralization strategy for over a hundred years against various bacterial toxins. Sometimes the old methods (or modern variants of!) prove to be best, even in light of rapidly evolving technologies evident throughout the biological sciences. It is known that recurring bouts of TSST-1 can be linked to low antibody titers, thus emphasizing the importance of host immune responses in controlling this life-threatening disease. In fact, low antibody titers to various S. aureus exotoxins (i.e., α and δ hemolysins, Panton–Valentin leukocidin, and SEC1) can also portend susceptibility to S. aureus sepsis in hospital patients. Furthermore, IVIg can be effective against staphylococcal-induced shock. These antibody preparations are derived from pooled human sera of those naturally hyperimmune to various S. aureus antigens. As S. aureus readily colonizes humans and grows (when given opportunity) in various consumed foods, seroconversion opportunities against various virulence factors (i.e., SEs and TSST-1) are many. With IVIg, there will naturally be many variables between lots (i.e., antibody recognition of different antigens, relative avidities, neutralizing capabilities, amounts of each antibody, etc). One way to minimize batch-to-batch variability of polyclonal antibodies is to develop human monoclonal antibodies characterized by various assays. Recent studies by different groups target select SEs, including SEB, with different types of antibodies (i.e., recombinant human or mouse–human chimeras) for various purposes (Table 4).
Table 5. Vaccine studies for staphylococcal superantigens

| Animal                  | Immunogen/adjuvant                                | Route  | Results                                                            |
|-------------------------|---------------------------------------------------|--------|-------------------------------------------------------------------|
| Mouse Balb/c            | SEB (N23K or F445 mutants)/aluminum hydroxide     | i.p.   | 80% protection against 30 LD₀₅₀ SEB challenge (i.p.) among vaccinated animals, vs. 7% protection for adjuvant-only controls. Sera from vaccinated mice protected naive animals against lethal SEB challenge.¹⁰³ |
| Mouse Balb/c            | SEB (L45R, Y89A, Y94A mutant)/aluminum hydroxide (i.p. route) or cholera toxin (i.n. and oral routes) | i.p., i.n., oral | Among i.p./i.n. vaccinated mice, there was 100% protection against either an 8 LD₀₅₀ (aerosol) or 30 LD₀₅₀ (i.p.) SEB challenge. Oral vaccination yielded 38% and 75% protection rates toward an ip or aerosol challenge, respectively. Only 10–10% of adjuvant-only controls were protected against either SEB challenge.²⁴,²⁹ |
| Mouse Balb/c            | TSST-1 (H135A mutant)/aluminum hydroxide          | s.c.   | Lethal S. aureus (i.v.) challenge resulted in 0% survival among adjuvant-only controls, vs. 60% protection for H135A-vaccinated animals.⁷⁷ |
| Mouse Balb/c            | TSST-1 (H135A mutant)/RIBI                         | i.p.   | Among the H135A-vaccinated animals, 67% were protected against a 15 LD₀₅₀ challenge (i.p.) of TSST-1 vs. 8% for adjuvant-only controls.²⁵ |
| Mouse NMRI             | SEA (L48R, Y92A, D70R mutant)/Freund's            | s.c.   | Vaccinated mice challenged with S. aureus (i.v.) had a delayed time to death and decreased weight loss, vs. BSA- vaccinated controls. Hyperimmune serum protected naive animals.⁵⁴ |
| Mouse transgenic for human HLA-DR3 and CD4 | SEB (L45R, Y89A, Y94A mutant)/RIBI | i.p.   | 100% protection against a 10 μg SEB challenge (i.p.) and markedly decreased IFN/IL-6 levels in vaccinated, vs. adjuvant-control, animals.¹⁰⁰ |
| Monkey Rhesus          | SEB (L45R, Y89A, Y94A mutant)/aluminum hydroxide  | i.m.   | A 20 μg dose given three times protected against SEB-induced hyperthermia, unlike adjuvant-only controls.¹⁰⁴ |
| Crossbred piglets       | SEB (L45R, Y89A, Y94A mutant)/Cholera toxin       | oral   | No ill effects with vaccine. Toxin-specific serum IgG and fecal IgA detected but cholera toxin did not enhance antibody response. No efficacy challenge results.¹⁰⁴ |

i.m., intramuscular; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal; i.v., intravenous; s.c., subcutaneous.

Reportedly much more thermal stable than “traditional” antibodies from other mammals. This unique attribute of camels, found also in sharks, could be quite useful in situations where a “cold chain” is not available for preserving reagents. Fab fragments (single-chain mouse) against SEB derived from phage display can also be useful, namely for diagnostic purposes, as host reactivity against heterologous species can be an issue.¹⁵⁰ Overall, these recombinant antibody-based approaches seem sage, as it is possible that one (or more) antibodies can be used to target one (or more) SEs as fully characterized reagents vs. relatively uncharacterized polyclonal preparations. It would be interesting to ascertain the ability of these, and other, monoclonal antibodies to neutralize natural variants of SEB and other SEs.¹⁵¹ Due to evolution and natural genetic drift, molecular variants of protein toxins (and other virulence factors) should always be considered for efficacy testing with any potential vaccines and therapeutics.

In addition to therapeutics, various groups have also developed different experimental vaccines for the staphylococcal superantigens. This approach for protection is logical, as the use of IVIg has also proven useful in humans following the onset of toxic shock.²⁸,²⁹,¹⁴⁶,¹⁴⁷ Experimentally, passive transfer of SEB-specific antibodies to naïve monkeys up to 4 h after an SEB aerosol also prevents lethal shock.¹⁵² Recombinantly attenuated mutants of SEA, SEB, and TSST-1 that do not bind MHC II and/or Vβ TCR molecules represent successful, experimental vaccines for preventing toxic shock in different animal models.²²-²⁷,¹⁰⁹,¹⁵³,¹⁵⁴ When given either parenterally or mucosally, these vaccines do not cause ill-effects and are efficacious against a toxin challenge or S. aureus infection (Table 5).

The Summary

S. aureus produces various superantigens representing important virulence factors that interact with MHC II, TCR, and accessory molecules on host cells. The host’s abnormally elevated immune response toward SEs or TSST-1 via various proinflammatory cytokines can trigger severe illness and lethal shock. Similar sequence homologies, structural conformations, and biological activities among this family of protein exotoxins suggest a common pathway through divergent and/or convergent evolution. More of these fascinating proteins, not only from S. aureus but also other microbes, will no doubt be discovered and novel biological properties likely elucidated by future investigators. Different assays, and more refinement of those that exist to date, will help lead the way to further discovery of much needed therapeutics and vaccines. There is an inherent urgency for more work in this field that extends well beyond biodefense. Bacterial pathogens like S. aureus, with wide dissemination among various mammalian species, multiple virulence factors, and increasing antibiotic resistance will be an omnipresent problem for many years into the future. We simply have now few effective tools for efficiently countering this bacterium and its various toxins like the SEs and TSST-1. Society needs, and increasingly demands (rightly so!), more effective controls of toxigenic pathogens like S. aureus. The future is now.
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