# Staphylococcal and Streptococcal Superantigens: Basic Biology of Conserved Protein Toxins

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**Abstract:** *Staphylococcus aureus* and *Streptococcus pyogenes* are gram-positive bacteria that possess great pathogenic potential in humans, causing numerous maladies such as arthritis, cutaneous infections, endocarditis, enterocolitis, food poisoning, pharyngitis, pneumonia, rheumatic fever, surgical site infections, and toxic shock. These prevalent pathogens produce various virulence factors that include the staphylococcal enterotoxins (SEs), toxic shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic exotoxins (SPEs). Minute (picomolar) amounts of these structurally-similar "superantigens" (SAgs) elicit high levels of proinflammatory cytokines and chemokines that can induce fever, hypotension, and lethal shock. *In vitro* and *in vivo* models have provided important tools for studying the biological effects of, and potential vaccines plus therapeutics against, these related protein toxins. This review will delve into the known physical and biological properties of the SEs, TSST-1, and SPEs. The reader will hopefully derive a general appreciation of these wonderfully-complex, structurally-similar toxins produced by *S. aureus* and *S. pyogenes*.

**Keywords:** Staphylococcal enterotoxin (SE), streptococcal pyrogenic exotoxin (SPE), superantigen, toxic shock, food poisoning, animal models, vaccines, therapeutics.

## INTRODUCTION

*Staphylococcus aureus* and *Streptococcus pyogenes* are non-motile, facultative, β-hemolytic bacteria that readily colonize skin and various mucosal surfaces via numerous virulence factors [1-4]. Discerning physical features in broth cultures include grape-like clusters formed by staphylococci versus chains for the streptococci. On agar plates, *S. aureus* colonies are usually yellow or orange (due to membrane-associated carotenoids), whereas *S. pyogenes* colonies appear gray and generally smaller. Typical biochemical differences between these bacteria involve catalase production, benzidine reaction, acid production in sugar-containing (lactose, mannitol, ribose, or salicin) broths, and growth in 6.5% NaCl.

From a virulence perspective, and in addition to the staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) described in detail throughout this review, *S. aureus* also produces several other virulence factors, for example: capsule, catalase, coagulase, C3-like ADP-ribosyltransferase (extensively reviewed in another article found in this Bacterial Toxin supplement), hemolysin, hyaluronidase, leukocidin, protein A, and superoxide dismutase [5]. Generation of biofilms by *S. aureus*, induced by anaerobic conditions plus low iron concentrations, adds yet another degree of difficulty in human attempts at thwarting this bacterium and related species [6]. Due to access issues, *S. aureus* within biofilms of tissue or implanted medical devices prove particularly resistant to antibiotics and the host's immune system, versus planktonic bacteria. Additionally, thirty percent of healthy humans host *S. aureus* and nasal colonization represents a major risk factor for subsequent infections [7, 8].

A real problem for now, and the future, involves ever-increasing resistance of *S. aureus* against commonly used antibiotics like methicillin (MRSA or methicillin-resistant *S. aureus*) [9] and vancomycin (VRSA or vancomycin-resistant *S. aureus*) [10, 11]. Such trends toward antibiotic resistance among clinical isolates of *S. aureus* were first noted during the 1940's, shortly after penicillin's introduction to the general population [12]. In Canada, it is estimated that approximately one hundred million dollars are spent every year for managing antibiotic-resistant *S. aureus* in hospitals, and costs for the dairy industry are even higher [13, 14]. The United States spends nearly fourteen billion dollars a year to fight *S. aureus*, of which sixty percent of isolates from intensive care units are MRSA [15]. There is no doubt that *S. aureus* truly represents an important health and economic concern throughout the world, involving various sectors of our society [14-19]. In health-care settings, transmission of MRSA between humans most commonly occurs by physical contact with contaminated hands (with or without gloves) [20]. Community-acquired MRSA is spread by various ways and amongst diverse populations, such as: 1) athletes participating in contact sports (i.e. direct skin to skin contact); 2) individuals in cramped living quarters with others, which include prisoners; 3) military personnel; 4) diabetics; and 5) those sharing personal items (i.e. razors, towels, clothes, etc.) that contact the skin [21].

The SEs (A-U) are associated with a prevalent form of food poisoning throughout the world [22-24]. The first definitive report of human staphylococcal food poisoning was in 1914 after consumption of milk from a cow with *S. aureus*-induced mastitis. Typically, SE intoxication occurs

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after ingesting processed meats or dairy products (i.e. ice cream, cottage cheese, custard, cream-filled pastries, etc.) contaminated by *S. aureus* via improper handling. Upon storage at temperatures that enable bacterial growth, there can be one or more SEs produced as a metabolic byproduct. Released toxin(s), and not the bacterium itself, ultimately causes classic symptoms of staphylococcal food poisoning. Very low microgram quantities of consumed SE are sufficient to cause emesis plus diarrhea within approximately four hours, and one may still experience a general malaise twenty-four to seventy-two hours after ingestion [23, 25]. Food poisoning by SEs is rarely fatal among healthy individuals; however, children and the elderly do represent the highest-risk groups for severe sequelae. Additionally, many MRSA strains (hospital- or community-acquired) also produce various SEs [26].

In contrast to SEs and food poisoning, toxic shock syndrome (TSS) caused by *S. aureus* colonization with subsequent TSST-1 release was first reported in 1978 and subsequently linked to tampon usage [27-29]. Increased levels of protein, carbon dioxide, and oxygen, as well as neutral pH within the microenvironment of *S. aureus* bound to vaginal epithelium are implicated in TSST-1 production *in vivo* [22, 30, 31]. An early report erroneously describes TSST-1 as an enterotoxin designated as SEF [32]; however, homogeneous SEF (later renamed TSST-1) lacks enterotoxicity in non-human primates [33]. Symptoms of staphylococcal TSS are linked to altered immunity that includes: 1) elevated serum levels of proinflammatory cytokines and chemokines; 2) rash (diffuse with intense erythroderma and subsequent desquamation); 3) hypotension (< 90 mm Hg); 4) fever (> 38.9$^{o}$C); and 5) adverse effects upon three or more organ systems [1, 2, 28, 30]. Non-menstrual TSS is also attributed to other SEs, like SEB and SED, from *S. aureus* growth on other body sites [34, 35]. In fact, non-menstrual TSS is now more prevalent and leads to higher mortality rates versus menstrual cases [35]. Similar to TSST-1, absorption of SEB through the vaginal (as well as nasal and conjunctival) mucosa results in toxic shock symptoms in a mouse model [36].

From 1980-2005, the percent of women (United States – Minnesota) vaginally colonized by *S. aureus* has increased (twelve versus twenty-three percent, respectively) and the toxin profiles of these strains have become different [37]. Antibodies towards two of these toxins (SEG and SEI) are much more evident in women versus men, perhaps suggesting a possible role during vaginal colonization by *S. aureus* [38]. All TSS patients may suffer recurring bouts unless the offending strain is kept at both minimal growth and toxin release. Antibodies seemingly play an important role in patient susceptibility to TSST-1-induced TSS [39, 40]. Patients that do not seroconvert against the offending toxin are more likely to have relapses of menstrual TSS. Depending upon toxin concentrations, there are varying effects upon human B cells *in vitro* that include apoptosis and decreased (when used at 1000 pg TSST-1/ml) or enhanced (between 1 – 0.01 pg TSST-1/ml) levels of antibody synthesis [41]. Such findings further emphasize a need for vaccines towards not only TSST-1, but also other SEs that play a role in staphylococcal-induced illness [42-50].

A microbial relative of *S. aureus*, *S. pyogenes*, is a group A streptococcus as defined by classic carbohydrate-based serotyping developed by Rebecca Lancefield [51]. Normal niches for *S. pyogenes*, like *S. aureus*, include skin and mucosal surfaces such as the upper respiratory tract. In contrast to *S. aureus*, *S. pyogenes* is strictly a human pathogen [52]. *S. pyogenes* causes various diseases such as acute glomerulonephritis, cellulitis, impetigo, necrotizing fasciitis, pharyngitis, rheumatic fever, and scarlet fever [1, 53, 54]. *S. pyogenes* possesses potent virulence factors that include: 1) protein toxins (streptolysins O and S); 2) anti-phagocytic capsule and cell-wall based M protein; 3) fibronectin binding proteins; as well as 4) a serine-type protease that inactivates the C5a component of complement [54]. The M1 serotype is often linked to outbreaks and much of the gene diversity between *S. pyogenes* strains is attributed to prophage DNA, which includes macrolide (erythromycin) resistance [55]. As later described, *S. pyogenes* produces multiple streptococcal pyrogenic exotoxins or SPEs (A, C, G-M) that are also prophage-encoded and possess similar biological (superantigenic) effects as the aforementioned *S. aureus* SEs and TSST-1 [1, 2]. In particular, the SPEs are linked to streptococcal TSS during bouts of bacteremia, necrotizing fasciitis (most common route of *S. pyogenes*-induced TSS), or rheumatic fever [1, 56]. Like that for *S. aureus* TSST-1 and menstrual TSS, antibodies against SPEs and M protein also play an important role in protective immunity against invasive group A streptococcal infections [57]. Circulating toxin can cause a characteristic rash (i.e. scarlet fever) as a consequence of host-induced inflammatory mediators and dilated blood vessels. For example, scarlet fever is most prevalent in children following wound or upper respiratory infections by *S. pyogenes*, with SPEA-producing strains often the culprit followed by SPEC producers [58, 59].

The term "superantigen" (SAg) commonly describes the SEs, TSST-1, SPEs or any other microbial protein that activates specific T-cells at sub-picogram per ml levels *in vitro* [1, 2, 60, 61]. Typical SAg interactions with host cells differ from conventional antigens, in that the former: 1) directly bind outside the peptide-binding groove of major histocompatibility complex (MHC) class II; 2) exert biological effects without internalization and cellular processing; as well as 3) are not MHC class II restricted. For the latter, there are differences between types of class II molecules and effective presentation of toxin to T cells [1, 2, 60]. Recognition of a SAg - MHC class II complex by T cells through T-cell receptor (TCR) depends upon the variable region within a TCR β chain (Vβ), thus differing from the Vα-Vβ chain combination common for conventional peptide antigens [1, 2, 62]. Microbial SAgs are reportedly produced by various gram-positive plus gram-negative bacteria [63-69], viruses [70-74], and even fungi [75]. However, of all the SAgs found in the literature, those from staphylococci and streptococci are generally better studied and best fit the classic definition of a SAg. For this particular review, our use of SAg will be interchangeable with SE, TSST-1, and SPE. The widespread nature of these microbial proteins with similar biological effects is highly suggestive of a successful survival strategy shared throughout the biosphere.

## GENETICS AND STRUCTURES OF SAgs

The SEs, TSST-1 and SPEs are secreted, single-chain proteins (twenty-three to thirty kilodaltons each) that cluster into distinct, amino acid-based homology groups [1, 2, 60]. Genes for these staphylococcal and streptococcal toxins are encoded by plasmids, bacteriophage, or mobile genetic elements and typically expressed during late logarithmic, into stationary, phases of growth [1, 2, 59, 76]. Differential gene location and *in situ* release of SEs and TSST-1 from *S. aureus* evidently cause noticeable differences in neutralizing antibody responses toward these toxins [77]. Some of the *S. aureus* SAgs (SEG, SEI, and SE-like proteins M, N, O, U) not commonly associated with TSS are encoded by an enterotoxin gene cluster (*egc*) within a pathogenicity island. Often, strains producing *egc*-based SAgs are harmless commensals. Additionally, the *egc*-associated toxins are released during logarithmic growth *in vitro* (versus late stationary phase for non-*egc* toxins like SEA, SEB, SEC, TSST-1) and, relative to non-*egc* toxins, do not readily stimulate production of neutralizing antibodies amongst human carriers of *egc*-containing strains of *S. aureus* [77, 78]. Regulation, *in vitro* or *in vivo*, of *egc* and non-*egc* SAgs is not well understood.

Structural genes for the SPEs are found on the chromosome or integrated bacteriophages. Promiscuous transfer of SPE genes by phage occurs between different group A strains, as well as to groups C and perhaps G streptococci, thus logically leading to increased virulence among normally avirulent strains [79]. Like that for *S. aureus* SAgs, the regulation of SPE genes is also not well understood [1]. A global regulator gene (*nra* or negative regulator of group A streptococci) maximally expressed during early stationary phase affects SPEA, binding proteins for fibronectin and collagen, as well as a positive regulator gene *mga* (multiple gene regulator of group A) [80]. SAg production varies between strains of *S. pyogenes in vitro*, while a host factor(s) evidently plays a role in toxin synthesis *in situ*. For instance, synthesis of SPEC and DNase by *S. pyogenes* may be naturally enhanced during infection by an unknown, human-derived factor(s) from pharyngeal cells [81].

X-ray crystallography of the SEs, TSST-1, and SPEs reveals a conserved, globular conformation consisting of two tightly-packed domains containing β-sheet plus α-helix structures (Fig. **1**). The domains are separated by a conserved, amphipathic α-helix of the β-grasp fold forming a shallow groove that interacts with surface loops on TCR [82-85]. The amino terminus contains an OB-fold while the carboxy terminus has β-grasp domains. In terms of general evolutionary relationships, the OB-fold family of protein domains consists of a five-stranded, closed β-barrel that commonly uses the same face for ligand binding and as an active site [86]. The prominent β-sheet and open barrel-like structure of the β-grasp fold control domain function [87]. Structure-function studies provide additional clues regarding specific residues critical for binding to MHC class II and TCR [45, 49, 88-90]. Upon considering overall similarities among bacterial SAgs, protein surfaces that involve interactions with MHC class II tend to be most conserved [91]. Furthermore, these SAgs share antigenic structures as evidenced by cross-reactivity and neutralization with polyclonal and monoclonal antibodies [92-96].

## BINDING OF SAgs TO CELLS

Staphylococcal and streptococcal SAgs bind distinct MHC class II molecules with relatively high affinity ($K_d$ = $10^{-8}$ - $10^{-6}$ M) [1, 2]. The HLA-DR (human class II) molecule binds better to SEs and TSST-1, versus HLA-DP or -DQ, while the preferential binding of SPEA to HLA-transfected L-cells is HLA-DQ > -DR > -DP. Competitive binding studies reveal at least two different binding sites on MHC class II molecules for the SEs and TSST-1. Among the SEs, SEA has the highest affinity for HLA-DR mediated by two separate binding sites [97-99]. The higher affinity site on SEA is within the C-terminus and binds HLA-DR β chain in a $Zn^{2+}$ -dependent manner [98]. The second binding site of SEA for HLA-DR is similar to that for SEB and located within the N-terminus, which interacts with the α chain of HLA-DR [99]. Cross-linking of two MHC class II molecules by a SAg leads to cytokine expression in monocytes [100-103].

Although analogous N-terminal regions from SEB and TSST-1 bind to MHC class II [89, 90, 104], co-crystals of SEB or TSST-1 complexed to HLA-DR1 reveal distinct differences [101, 102]. Uniquely, SEB interacts exclusively with the α chain of HLA-DR1 and is unaffected by presentation of peptide antigen. TSST-1 binds to both α and

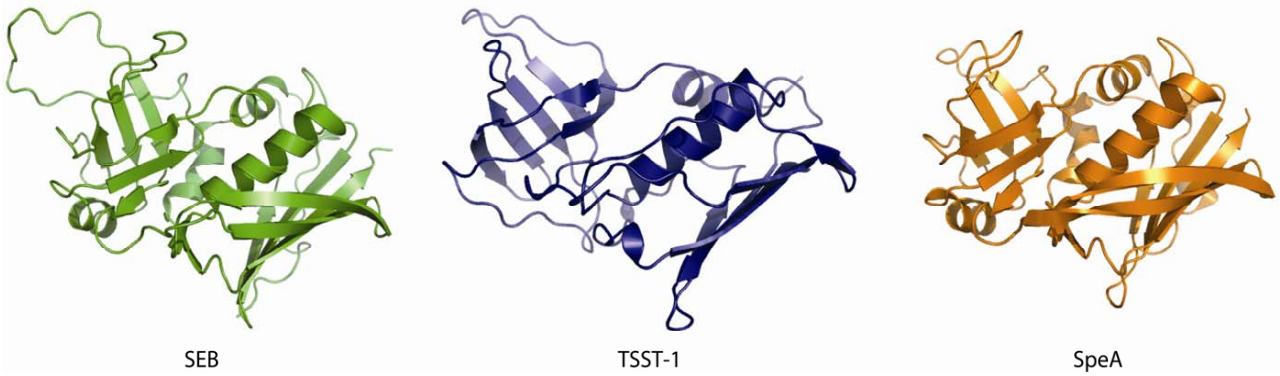


**Fig. (1).** Crystal structures of SEB [83] (Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) identification = 3SEB), TSST-1 [84] (PDB identification = 2QIL), and SPEA [85] (PDB identification = 1FNU) were generated with PyMol software (DeLano Scientific LLC, Palo Alto, CA).

β chains of class II molecules and is affected by residing peptide antigen. SPEA also differs from most other SPEs by binding to the α, not β, chain of MHC class II. A $Zn^{+2}$ molecule generally serves an important role in SPE binding and subsequent biological effects, with SPEC being an exception [1, 105]. Although SPEC uses $Zn^{+2}$ – dependent binding to MHC II, this is not essential for T-cell activation and suggests $Zn^{+2}$ – independent interactions [105]. The evolutionary similarity between streptococcal and staphylococcal SAgs is further evidenced in SPEH, as this "hybrid" toxin consists of an N-terminal domain related to the SEB homology group and a C-terminus resembling SPEC [88].

The groove formed between SE, TSST-1, or SPE domains represents an important binding site for TCR Vβ chain [83-85, 89, 90, 101]. These toxins typically bind (approximately $10^{-6}$ M affinity) to a distinct repertoire of Vβ-bearing T-cells, thus generating a "fingerprint" useful for diagnosing staphylococcal- and streptococcal-induced TSS [106-108]. The SAg - MHC class II complex binds to the carbon backbone, but not side-chain residues, of TCR Vβ [62, 109, 110]. There is one notable exception, as SEH binds to T cells via Vα interactions [111]. An additional unique characteristic of SEH is the sub-nanomolar affinity for MHC class II [112]. Depending upon the SAg, there are differences in how antigen presenting cells (APCs) and T cells interact [113]. For instance, SPEC or TSST-1 form a bridge between cells while the MHC class II and TCR molecules do not make direct contact. In contrast, SEB does not inhibit direct MHC class II – TCR interactions.

## HOST CELL RESPONSES TO SAgs

Recognition of the SAg - MHC class II complex by T cells results in signaling, profound proliferation, and ultimately cytokine production by APCs plus T cells [1, 2]. Peripheral blood mononuclear cells (PBMCs) from humans are commonly used *in vitro* to study cell activation by staphylococcal and streptococcal SAgs [114-119]. PBMCs secrete various cytokines / chemokines following SAg exposure, and these include interleukin (IL)-1, -2, -6, tumor necrosis factor (TNF) α, TNF β, interferon (IFN) γ, macrophage inflammatory protein 1α (MIP-1α), MIP-1β, and monocyte chemoattractant protein-1 (MCP-1). T-cell recognition of SAg, when bound to an APC through MHC class II, becomes the triggering event for cytokine and chemokine release [114, 120-122]. Contradictions exist in the literature though, regarding APC and T-cell responses to these toxins without the other cell type [114, 123]. MHC class II-based stimulation of T cells by SAgs is a general requirement, but cells possessing select TCR Vβ types (i.e. human 6, 7, and 18 with SEA) can also independently respond, albeit less efficiently [124]. Perhaps the MHC class II-dependent Vβ types require class II molecules for more effective cell activation to overcome lower binding affinity to TCR? Additionally, SAg (i.e. SEB) presentation to $V\beta3^{+}$ T-cells without MHC class II-bearing cells can also decrease TCR expression and induce anergy [125].

In addition to APCs and T cells, bacterial SAgs can also have both stimulatory and destimulatory effects upon B cells and synovial fibroblasts. For example, cross-linking of TCR with MHC class II triggers B-cell proliferation and immunoglobulin synthesis in a dose-dependent manner, but high concentrations of toxin actually inhibit antibody synthesis [41, 126]. Continual SE exposure during chronic rhinosinusitis linked to *S. aureus* colonization can lead to localized, toxin-specific IgE and shifting of IgG subclasses (i.e. elevated IgG4 and decreased IgG2 versus individuals without SE-specific IgE) [127]. Early exposure to SEs as a neonate, via colonization by toxinogenic *S. aureus*, may also play an important role in oral tolerance and food allergies [128]. Suppression of antibody secretion by TSST-1 occurs by inducing apoptosis among B cells [41], critically impacting humoral immunity against this toxin that can lead to recurring bouts of TSS [39, 40]. Direct stimulation of synovial fibroblasts by SAgs also induces chemokine expression, suggesting that autoreactivity and chemotactic responses could initiate or augment chronic inflammation such as rheumatoid arthritis [129-131]. Additionally, intestinal myofibroblasts respond directly to SEA (but not SEB) by producing MCP-1, IL-6, and IL-8 which is also an effect mimicked by cross-linking MHC class II molecules with antibody [132]. Overall, bacterial SAgs can have multiple effects upon various aspects of the host's immune system.

## *IN VIVO* EFFECTS OF SAgs

From a food poisoning perspective with SEs, specific cells and receptors in the intestinal tract have not been identified unequivocally. Data suggest that leukotrienes play a role in SEB-induced emesis and skin reactions (non-human primate model) while serotonin binding to vagal afferent neurons plus the cannabinoid receptor 1 affect SEA-induced emesis (shrew model) [133, 134]. The SEs readily induce emesis in primates (human and non-human) upon ingesting low microgram quantities, while larger toxin amounts can lead to toxic shock [23, 135]. Studies with SEs and non-human primates are considered the gold standard for *in vivo* work linked to food poisoning; however, these efforts are increasingly more expensive (politically and monetarily) and thus fuel exploration by various laboratories for alternative animal models (Table **1**). Typically, TSST-1 or SPEs do not cause vomiting after ingestion although they do naturally cause TSS in humans and animals [1, 2, 33, 136]. An oral dose of SEB activates $V\beta8^{+}$ T-cells in Peyer's patches of mice, increasing IFNγ and IL-2 mRNA expression [137]. Either SEA or SEB alone causes intestinal inflammation, but these toxins can also exacerbate a microbe-based syndrome called inflammatory bowel disease which suggests an immune response to ingested SEs [138]. An enteric immune connection may also explain fifty year-old results published by Sugiyama *et al*. [139], as they discovered that non-human primates given intragastric SE become transiently resistant to another dose of the same (but not a different) SE. Such findings are likely linked to toxin-specific stimulation of unique Vβ-bearing T-cells that subsequently become anergic.

To further understand how SE ingestion affects intestinal mucosa, *in vitro* studies with human Caco-2 monolayers reveal transcytosis (albeit by different mechanisms) of SEA, SEB, and TSST-1 [140]. In this model system, there is facilitated transport of SEB and TSST-1 in a bidirectional fashion (i.e. basolateral to apical or *vice versa*). Transport of

**Table 1. Toxic Shock Models for Staphylococcal and Streptococcal SAgs**

| Animal (strain) | Agent(s) | Reference(s) |
|---|---|---|
| Mouse (Balb/c) | TSST-1 + LPS | [42, 153, 165] |
| Mouse (C57/BL6) | SEA or SEC1 + LPS | [162] |
| Mouse (Balb/c) | SEB + LPS | [153, 171, 187] |
| Mouse (Balb/c) | SEB + D galactosamine | [156] |
| Mouse (Balb/c) | SEB + Actinomycin D | [163] |
| Mouse (CBA) | SEB + Virus | [164] |
| Mouse (C3H/HeJ) | SEB | [166] |
| Mouse (Transgenic HLA-DQ8 + CD4) | SPEA | [185] |
| House Musk Shrew | SEA, SEE, or SEI | [202, 203] |
| Rat (Sprague-Dawley) | SEB + LPS | [170] |
| Ferret | SEB | [201] |
| Rabbit (Dutch Belted) | TSST-1 + LPS | [165] |
| Rabbit (Dutch Belted) | SEC +LPS | [172] |
| Rabbit (Dutch Belted) | SPEA or SPEC | [195] |
| Rabbit (New Zealand White) | SEA | [194] |
| Cat | SEA | [224] |
| Goat (Dwarf) | SEB or TSST-1 | [200] |
| Monkey (Rhesus) | SEB | [213, 217] |

SEA is in contrast less rapid and non-specific. Mutant forms of SEB altered at residues Asn23Lys or Phe44Ser, which respectively decrease toxin binding to TCR and MHC class II, also decrease transcytosis by fifty to seventy percent. In this same report, additional studies with mice show that ingested SEB enters the circulatory system (peak levels appear approximately two hours after ingestion) at much higher concentrations (twenty-fold) than SEA. The SEs do not act as direct cytotoxins upon human intestinal cells [141], but they do disturb gut mucosa by increasing ion permeability in the presence of SE-stimulated PBMCs and cytokine / chemokine release *in vitro* [142].

Perhaps in contrast to the above transcytosis results, SE binding to MHC class II may not play a role in enterotoxicity. Attenuated variants of SEA (Leu48Gly) and SEB (Phe44Ser), with diminished binding to MHC class II molecules and decreased stimulation of T cells, remain emetic [143]. Although carboxymethyl modification of histidines on SEA [144] and SEB [145] minimally affect superantigenicity, these molecules lack enterotoxicity, lethal effects, and skin reactivity [133, 146, 147]. In non-human primates, this modified SEB inhibits vomiting and diarrhea of wild-type SEB when given together, perhaps suggesting competition for a common receptor(s) in the gut [146]. Individual recombinant changes of His44, His50, His114, or His187 on SEA to either alanine or aspartic acid have no effect upon biological activity, yet modification of His225 impacts both emesis and superantigenicity [147].

Besides toxin-specific resistance after an oral dose of SE, chronic intravenous exposure functionally inactivates all Vβ-reactive T-cells in mice [148]. Footpad injections of SEB in mice also elicit a dose-related tolerance within sixteen hours among circulating $V\beta8^{+}$ T-cells [149]. Subcutaneous exposure to low doses of SEB in mice causes a general immunosuppression inhibited by IL-12 [150]. IL-12, produced by dendritic cells and macrophages, plays many roles that include differentiation of T cells. Additionally, immunosuppression induced by repeated exposure to SEB is also linked to activation of regulatory T-cells ($Foxp^{3+}$ plus $CD152^{high}$) and catabolism of tryptophan by indoleamine-2,3-dioxygenase [151].

Similar to SEB, mice given a microgram of SEA intranasally (once a week for three weeks) also become resistant to a subsequent lethal challenge of SEA, but not TSST-1 [152]. Such tolerance is not due to toxin-specific antibody or functional deletion of SEA-reactive T-cells. However, increased IL-10 levels in sera from these animals correlate with other findings showing that this cytokine protects against SE or TSST-1 intoxication [153, 154]. T-cell studies of patients with rheumatic fever (acute/chronic), or healthy donors, show varying degrees of stimulation (or lack of) by SPEA *in vitro* [155]. Overall, dampening of host immunity is a general characteristic of the staphylococcal and streptococcal SAgs which possibly enhances pathogen survival.

SAgs perturb the immune system by inducing high levels of proinflammatory cytokines in various animals that include primates, rabbits, and mice (Table **1**) [1, 2, 23, 46, 47, 116-118, 156-159]. IL-1 and TNFα are endogenous pyrogens that induce fever via hypothalamus release of prostaglandin E2 [160]. Circulating levels of IFNγ, IL-2, and IL-6 also increase after toxin exposure. IFNγ particularly feeds into SAg-fueled toxicity by increasing MHC class II expression among various cell types (i.e. APCs, epithelial cells, and endothelial cells), plus augmenting the proinflammatory actions of IL-1 and TNFα. SAg-driven TSS results from a cacophony of released cytokines plus chemokines that adversely affect critical organs throughout the body [1, 2].

Mice represent an alternative model to large vertebrates (i.e. non-human primates or rabbits) for studying SAg-mediated shock (Table **1**) [156, 161-166]. Relative to non-

human primates, mice are a very viable option for basic toxin studies involving potential vaccines and therapeutics. However, mice lack an emetic response and are thus less appropriate for studying certain aspects of food poisoning due to SEs. Mice are also naturally less susceptible than primates to SAgs, largely because of decreased affinity for murine MHC class II [1, 2]. Various groups have shown that potentiating agents like D-galactosamine, actinomycin D, lipopolysaccharide (LPS), viruses (influenza and lymphocytic choriomeningitis), or even pathogenic protozoa (*Trypanosoma cruzi*, causative agent of Chagas disease) greatly amplify SAg toxicity and TSS effects in mice [161-168]. The natural synergy between SAgs with diverse pathogens, including outer membrane-based LPS of gram-negative bacteria, provides yet additional intrigue for truly understanding complex microbe-host interactions.

For many years our laboratory has favored a LPS-potentiated model in mice, as various studies show a natural synergy existing between SAgs and LPS [153, 161, 162, 167, 169-173]. Very minute (nanogram) quantities of LPS alone in humans elevate cytokine levels, temperature, and heart rate [174], yet SAgs like the SEs, TSST-1, and SPEs can dangerously augment LPS effects many log-fold [157]. Upon considering the sheer number of gram-negative bacteria that make up intestinal flora, along with a recognized increase of such bacteria among TSS patients, the probability of SAg(s) and LPS synergy is rather high [157, 175]. There is a correlation between elevated serum levels of various proinflammatory cytokines (IL-1, IL-2, TNFα, IFNγ) with severity of SEA-, SEB-, or TSST-1-induced TSS [2, 42, 61, 156, 162]. Further studies with knockout mice show that IL-10 affects various cytokine and chemokine (MIP-1α and MIP-2) levels in serum following SEB intoxication [176]. Two different receptors for TNFα play differential roles in SE-induced shock, as p55 (type 1) serves a more prominent role versus p75 (type 2) [153]. Various cell markers such as CD28 (also known as TP44), CD43 (leukosialin), CD54 (intercellular adhesion molecule-1 or ICAM-1), and CD95 (Fas or cell death receptor) also have prominent functions in SAg-induced shock *in vivo* [177-182].

An additional twist to using mice for studying staphylococcal and streptococcal SAgs involves transgenics expressing human receptors, such as HLA-DQ6 and CD4. These animals succumb to normally sublethal amounts of SEB (with D-galactosamine potentiation), and the serum levels of TNFα correlate with onset of lethal shock [183]. Transgenics expressing human HLA-DR3 and CD4 lethally respond to a SE challenge without a potentiating agent, thus providing a more easily-interpreted model [184]. When naïve PBMCs from these transgenics are incubated with SEB *in vitro*, IL-6 and IFNγ release is markedly elevated versus that from cells of control (non-transgenic) BALB/c mice. As reported from other animal models for staphylococcal and streptococcal SAgs, these results with HLA-DR3/CD4 mice suggest involvement of proinflammatory cytokines. Similar studies using transgenic mice, with similar results, have also been reported using SPEA and mice expressing human HLA-DQ8 plus CD4 [185]. Additionally, transgenics over-expressing TCR Vβ3 (murine) experience increased mortality from elevated TNF and IFNγ levels during infection by SEA-producing *S. aureus* [186]. Mice expressing human HLA and CD4, or even increased levels of specific murine TCR, will likely provide a clearer understanding of SAg-mediated effects *in vivo* without potentiating agents.

Besides lethality, temperature represents an important parameter for studying SE- or TSST-1-induced shock in various animal models (Table **1**). In mice, these studies can be accomplished by implanting a subcutaneous transponder [153] or intraperitoneal telemetry device [187]. Results reveal a rapid (less than 10 hours after toxin challenge) temperature decrease that provides a quick, non-lethal parameter for intoxication. Mouse studies have not unveiled a temperature increase following intoxication by a SE, in contrast to non-human primates given SEB [188], thus suggesting a very rapid onset of SE-induced TSS in mice and subtle differences between models.

In addition to overt signs of SAg intoxication *in vivo* (i.e. temperature, emesis, diarrhea, and death), SEB induces apoptosis and decreased signal transduction among specific Vβ-bearing T-cells due to loss of L-selectin (a cell adhesion molecule) [189, 190]. Surface levels of TCR-CD3 also decrease approximately fifty percent among Vβ-reactive T-cells within just thirty minutes after SEB exposure [191]. T-cell proliferation in mice following an SEB injection is transient, as within forty-eight hours the majority of activated T-cells undergo apoptosis [61]. After two intraperitoneal injections (one hundred micrograms each) of SEB into mice, splenic $V\beta8^+$ T-cells become non-responsive to SEB *in vitro*. These cells produce less IL-2, but more IFNγ and IL-10, within forty-eight hours after the initial toxin dose [192]. In contrast to elevated levels of proinflammatory IFNγ, anti-inflammatory IL-10 perhaps reflects an attempt by the host to counter deleterious effects of other cytokines and chemokines.

In addition to numerous studies with mice (Table **1**), rabbits have also afforded a reliable *in vivo* model for SAg-induced TSS although little information exists regarding SAg interactions with rabbit MHC class II and TCR [165, 172, 193-198]. For rabbits, use of an implanted infusion pump to slowly release toxin into the body mimics a natural setting during *S. aureus* or *S. pyogenes* infection [193, 195]. Variation in strain susceptibility to a specific toxin exists in rabbits (New Zealand White versus Dutch-Belted), just like that discovered in mice [153, 196]. Similar to humans, rabbits exposed to either TSST-1 or SEB develop elevated serum levels of LPS eliminated by polymyxin B, along with diminished clinical signs of TSS [169, 196, 197]. Higher concentrations of circulating LPS may be due to impaired liver clearance [172, 198], further exacerbated by SAgs like the SEs, TSST-1, or SPEs [170, 199].

Other less-defined animal models for SAg intoxication have been described in the literature (Table **1**). One example includes goats used for TSST-1 and SEB, with fever as a measurable parameter after intravenous administration [200]. There is also a reported ferret model for oral intoxication that employs relatively large (milligram) quantities of SEB, using emesis and fever as markers [201]. Another emetic model for the SEs employs the rather unusual house musk shrew challenged intraperitoneally or orally [202]. Recently, this latter model has been used successfully for vaccine studies with recombinantly-attenuated SEA [203]. An obvious, and

less than pleasant, caveat with any emetic (or diarrheic) model involves quantitation.

Although basic aspects of intoxication have been investigated in each animal model listed above (Table **1**), critical work still remains for vaccine and therapeutic discovery. Any model (*in vitro* or *in vivo*!) has positive, but also negative, aspects that require any investigator to closely scrutinize data and interpret usefulness of such information for human application.

## THERAPEUTICS AND VACCINES AGAINST SAgs

Other than a precarious societal reliance upon antibiotics, which continue to become less practical with rising microbial resistance, there are surprisingly few clinically-useful options for therapeutics and there are no approved vaccines targeting the staphylococcal and streptococcal SAgs. To circumvent SAg stimulation, potential therapies and vaccines against these toxins should interfere with one or more of the following: 1) TCR – SAg – MHC class II interactions; 2) accessory, co-stimulatory, or adhesion molecules necessary for T-cell activation; and 3) proinflammatory cytokine plus chemokine release from host cells.

Several of the aforementioned models (*in vitro* and *in vivo*) have been used to study potential therapies against SAg-induced TSS. As just a few examples that uniquely attack SAg-induced TSS, inhibitors of nitric oxide synthase (i.e. aminoguanidine or dexamethasone) can diminish SEA-induced IL-1, -2, -6, TNF, and IFNγ production from human PBMCs *in vitro*, as well as prevent neutrophil influx into rat lungs *in vivo* [204, 205]. The blocking of CD28 co-stimulatory receptor by a synthetic ligand, CTLA4-Ig, prevents TSST-1-induced effects *in vitro* and *in vivo* [206]. Antibodies against TNFα prevent SEB-induced lethality [156], while IL-10 blocks IL-1, TNFα, as well as IFNγ synthesis that then reduces TSS symptoms [154]. Studies with human PBMCs *in vitro* and a mouse model show that either pentoxifylline or pirfenidone (drugs that diminish proinflammatory cytokine synthesis) effectively decreases SEB or TSST-1 toxicity [116, 117]. Tryptanthrin, naturally derived from a medicinal plant, is an anti-inflammatory alkaloid that inhibits cyclooxygenase 2 (COX2) and decreases IFNγ release from SEB-stimulated lymphocytes in Peyer's patches [207]. A hexapeptide (anti-leukinate) inhibitor of IL-8, a cytokine which elicits SEA-induced inflammation of the lungs, decreases neutrophil influx and subsequent lung damage [208]. Finally, soluble antagonists for TCR Vβ have also proven effective *in vitro* and *in vivo* (rabbit model) against SEB [209]. These reagents were generated from a murine Vβ8.2 clone that was affinity matured into small (twelve kilodalton), higher-affinity (six log lower $K_d$ versus wild-type) molecules through random mutagenesis.

Besides the aforementioned experimental therapeutics, different antibody-based therapies and experimental vaccines that directly target SAgs have been developed over many decades. Preexisting antibodies toward these toxins play an important role in disease outcome [39, 40, 210], and intravenous immunoglobulin (IVIG) has been useful following the onset of TSS due to *S. aureus* or *S. pyogenes* infection [211, 212]. From a bioterror perspective involving an aerosol of SEB, passive transfer of SEB-specific antibodies to naïve rhesus monkeys within four hours after exposure prevents TSS [213]. Similar results were discovered earlier in mice, suggesting important parallels between diverse animal models for aerosol or intraperitoneal administration of SEB [214]. Recombinantly-attenuated forms of staphylococcal and streptococcal SAgs that ineffectively bind MHC class II and/or specific Vβ TCR represent promising vaccines against TSS [42-50, 203, 215-217]. When given either parenterally [44, 45, 50, 203], or mucosally [43, 218], such vaccines are effective against a toxin challenge or *S. aureus* infection; however, mucosal vaccination with these toxin-based immunogens has remained largely unexplored. In addition to recombinant vaccines for SAgs, dated literature reveals that formaldehyde toxoids of SEA, SEB, or SEC1 also represent effective parenteral or mucosal immunogens [219, 220]. Although chemically-generated toxoids of the SEs and many other toxin antigens exist, such treatment can adversely affect native epitopes, antigen processing, and subsequent presentation to the immune system [221, 222]. In our opinion, intimate knowledge of how a toxin interfaces with receptor (i.e. co-crystal or structure/function data) leads to minimal manipulations of the toxin into an immunogen that best retains proper conformation and epitopes.

## CONCLUSIONS

*S. aureus* and *S. pyogenes* are very formidable pathogens that cause a wide array of diseases, including TSS [113]. One common, protein-based aspect these bacteria share involves synthesis of toxic SAgs. Intoxication by SEs, TSST-1, or SPEs occurs through the host's abnormal response to the offending toxin (i.e. elevated proinflammatory cytokine / chemokine levels), that can trigger shock and possibly death. After cytokine and chemokine release, ensuing imbalance of the immune system likely aids survival of the offending pathogen [223]. Similar sequence homologies, conformations, and biological activities amongst the staphylococcal and streptococcal SAgs suggest common evolution as well as a common means of medicinally thwarting their intoxication process. New SAgs from *S. aureus* and *S. pyogenes* are constantly being discovered by groups from around the world. More effective controls targeting *S. aureus*, *S. pyogenes*, plus their associated toxins are necessary and include new therapeutics as well as vaccines. Many of the latter forms of medicine remain experimental, with promise for the not so distant future. In particular, human monoclonal antibodies that target SAgs and are well characterized could be quite efficacious as well as afford distinct advantages versus IVIG.

Finally, the constant evolution of pathogens like *S. aureus* and *S. pyogenes* requires that humans co-evolve towards recognizing and then effectively challenging these constantly changing, and quite deadly, microbial threats. The SEs, TSST-1, and SPEs portrayed in this review represent just one piece of a complex puzzle towards better management of staphylococcal and streptococcal infections.

## REFERENCES

[1] Proft T, Fraser JD. In: Alouf JE, Popoff MR, Eds. The comprehensive sourcebook of bacterial protein toxins. Paris: Academic Press 2006; pp. 844-61.

[2] Uchiyama T, Imanishi K, Miyoshi-Akiyama T, Kato H. In: Alouf JE, Popoff MR, Eds. The comprehensive sourcebook of bacterial protein toxins. paris: Academic Press 2006; pp. 830-43.
[3] Tristan A, Ferry T, Durand G, *et al.* Virulence determinants in community and hospital methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 2007; 65: 105-9.
[4] Krause RM. A half-century of streptococcal research: then and now. Indian J Med Res 2002; 115: 215-41.
[5] DeLeo FR, Diep BA, Otto M. Host defense and pathogenesis in *Staphylococcus aureus* infections. Infect Dis Clin N Am 2009; 23: 17-34.
[6] Gotz F. *Staphylococcus* and biofilms. Mol Microbiol 2002; 43: 1367-78.
[7] Graham PL, Sin SX, Larson EL. A US population based survey of *Staphylococcus aureus* colonization. Ann Intern Med 2006; 144: 318-25.
[8] Wertheim HF, Melles DC, Vos MC, *et al.* The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis 2005; 5: 751-62.
[9] Leclercq R. Epidemiological and resistance issues in multidrug-resistant staphylococci and enterococci. Clin Microbiol Infect 2009; 15: 224-31.
[10] CDC. Morb Mortal Weekly Rep 1997; 46: 624-6.
[11] CDC. Morb Mortal Weekly Rep 2002; 51: 565-7.
[12] Barber M, Rozwadowska-Dowzenko M. Infection by penicillin-resistant staphylococci. Lancet 1948; 1: 641-4.
[13] Lang A, Ward S, Michie CA. In brief. Trends Immunol 2002; 23: 67-8.
[14] Goetghebeur M, Landry P-A, Han D, Vicente C. Methicillin-resistant *Staphylococcus aureus*: a public health issue with economic consequences. Can J Infect Dis Med Microbiol 2007; 18: 27-34.
[15] Noskin GA, Rubin RJ, Schentag JJ, *et al.* National trends in *Staphylococcus aureus* infection rates: impact on economic burden and mortality over a 6 year period (1998-2003). Clin Infect Dis 2007; 45: 1132-40.
[16] Gonano M, Hein I, Zangerl P, Rammelmayr A, Wagner M. Phenotypic and molecular characterization of *Staphylococcus aureus* strains of veterinary, dairy and human origin. Epidemiol Infect 2008; 137: 688-99.
[17] Shorr AF, Micek ST, Kollef MH. Inappropriate therapy for methicillin-resistant *Staphylococcus aureus*: resource utilization and cost implications. Crit Care Med 2008; 36: 2335-40.
[18] DeCock E, Krueger WA, Sorensen S, *et al*. Cost-effectiveness of linezolid vs vancomycin in suspected methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia in Germany. Infection 2009; 37: 123-32.
[19] Capitano B, Leshem OA, Nightingale CH, Nicolau DP. Cost effect of managing methicillin-resistant *Staphylococcus aureus* in a long-term care facility. J Am Geriatr Soc 2003; 51: 10-16.
[20] McBride ES, Bradley LC, Whitby M, McElwain DL. An investigation of contact transmission of methicillin-resistant *Staphylococcus aureus*. Hosp Infect 2004; 58: 104-8.
[21] Miller LG, Kaplan SL. *Staphylococcus aureus*: a community pathogen. Infect Dis Clin N Am 2009; 23: 35-52.
[22] Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev 2000; 13: 16-34.
[23] Bergdoll MS. Monkey feeding test for staphylococcal enterotoxin. Meth Enzymol 1988; 165: 324-33.
[24] Loir L, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. Genet Mol Res 2003; 31: 63-76.
[25] Bennett RW. Staphylococcal enterotoxin and its rapid identification in foods by enzyme-linked immunosorbent assay-based methodology. J Food Prot 2005; 68: 1264-70.
[26] Naimi TS, LeDell KH, Como-Sabetti K, *et al.* Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 2003; 290: 2976-84.
[27] Todd J, Fishaut M, Kapral F, Welch T. Toxic-shock syndrome associated with phage-group-I staphylococci. Lancet 1978; 2: 1116-8.
[28] Shands KN, Schmid GP, Dan BB, *et al.* Toxic-shock syndrome in menstruating women: association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases. N Engl J Med 1980; 303: 1436-42.
[29] Schlievert PM, Shands KN, Dan BB, Schmid GP, Nishimura RD. Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic-shock syndrome. J Infect Dis 1981; 143: 509-16.
[30] McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. Ann Rev Microbiol 2001; 55: 77-104.
[31] Mills J, Parsonnet J, Tsai YC, Kendrick M, Hickman RK, Kass EH. Control of production of toxic-shock-syndrome toxin-1 (TSST-1) by magnesium ion. J Infect Dis 1985; 151: 1158-61.
[32] Bergdoll MS, Crass BA, Reiser RF, Robbins RN, Davis JP. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock-syndrome *Staphylococcus aureus* isolates. Lancet 1981; 1: 1017-21.
[33] Reiser RF, Robbins RN, Khoe GP, Bergdoll MS. Purification and some physicochemical properties of toxic-shock toxin. Biochemistry 1983; 22: 3907-12.
[34] Andrews MM, Parent EW, Barry M, Parsonnet J. Recurrent nonmenstrual toxic shock syndrome: clinical manifestations, diagnosis, and treatment. Clin Infect Dis 2001; 32: 1470-9.
[35] Descloux E, Perpoint T, Ferry T, *et al.* One in five mortality in non-menstrual toxic shock syndrome versus no mortality in menstrual cases in a balanced French series of 55 cases. Eur J Clin Microbiol Infect Dis 2008; 27: 37-43.
[36] Rajagopalan G, Smart MK, Murali N, Patel R, David CS. Acute systemic immune activation following vaginal exposure to staphylococcal enterotoxin B-implications for menstrual shock. J Reprod Immunol 2007; 73: 51-9.
[37] 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, 2005. J Clin Microbiol 2007; 45: 2704-7.
[38] Banks MC, Kamel NS, Zabriskie JB, Larone DH, Ursea D, Posnett DN. *Staphylococcus aureus* express unique superantigens depending on the tissue source. J Infect Dis 2003; 187: 77-86.
[39] Bonventre PF, Linnemann C, Weckbach LS, *et al*. Antibody responses to toxic-shock-syndrome (TSS) toxin by patients with TSS and by healthy staphylococcal carriers. J Infect Dis 1984; 150: 662-6.
[40] Vergeront JM, Stolz SJ, Crass BA, Nelson DB, Davis JP, Bergdoll MS. Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: implications for toxic-shock syndrome. J Infect Dis 1983; 148: 692-8.
[41] Hofer MF, Newell K, Duke RC, Schlievert PM, Freed JH, Leung DY. Differential effects of staphylococcal toxic shock syndrome toxin-1 on B cell apoptosis. Proc Nat Acad Sci USA 1996; 93: 5425-30.
[42] Stiles BG, Krakauer T, Bonventre PF. Biological activity of toxic shock syndrome toxin 1 and a site-directed mutant, H135A, in a lipopolysaccharide-potentiated mouse lethality model. Infect Immun 1995; 63: 1229-34.
[43] Stiles BG, Garza AR, Ulrich RG, Boles JW. Mucosal vaccination with recombinantly attenuated staphylococcal enterotoxin B and protection in a murine model. Infect Immun 2001; 69: 2031-6.
[44] Nilsson IM, Vergrengh M, Ulrich RG, Bavari S, Tarkowski A. Protection against *Staphylococcus aureus* sepsis by vaccination with recombinant staphylococcal enterotoxin A devoid of superantigenicity. J Infect Dis 1999; 180: 1370-3.
[45] Bavari S, Dyas B, Ulrich RG. Superantigen vaccines: a comparative study of genetically attenuated receptor-binding mutants of staphylococcal enterotoxin A. J Infect Dis 1996; 174: 338-45.
[46] McCormick JK, Tripp TJ, Olmsted SB, *et al*. Development of streptococcal pyrogenic exotoxin C vaccine toxoids that are protective in the rabbit model of toxic shock syndrome. J Immunol 2000; 165: 2306-12.
[47] Roggiani M, Stoehr JA, Olmsted SB, *et al*. Toxoids of streptococcal pyrogenic exotoxin A are protective in rabbit models of streptococcal toxic shock syndrome. Infect Immun 2000; 68: 5011-7.
[48] Gampfer J, Thon V, Gulle H, Wolf HM, Eibl MM. Double mutant and formaldehyde inactivated TSST-1 as vaccine candidates for TSST-1-induced toxic shock syndrome. Vaccine 2002; 20: 1354-63.

[49] Ulrich RG, Olson MA, Bavari S. Development of engineered vaccines effective against structurally related bacterial superantigens. Vaccine 1998; 16: 1857-64.

[50] Hu DL, Omoe K, Sasaki S, *et al*. Vaccination with nontoxic mutant toxic shock syndrome toxin-1 protects against *Staphylococcus aureus* infection. J Infect Dis 2003; 188: 743-52.

[51] Lancefield RC. The antigenic complex of *Streptococcus haemolyticus*. I. Demonstration of a type-specific substance in extracts of *Streptococcus haemolyticus*. J Exp Med 1928; 47: 91-103.

[52] Bessen DE. Population biology of the human restricted pathogen, *Streptococcus pyogenes*. Infect Genet Evol 2009; 9: 581-93.

[53] Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis 2005; 5: 685-94.

[54] Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 2000; 13: 470-511.

[55] Banks DJ, Beres SB, Musser JM. The fundamental contribution of phages to GAS evolution, genome diversification and strain emergence. Trends Microbiol 2002; 10: 515-21.

[56] Stevens DL, Tanner MH, Winship J, *et al*. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. N Engl J Med 1989; 321: 1-7.

[57] Basma H, Norrby-Teglund A, Guedez Y, *et al*. Risk factors in the pathogenesis of invasive group A streptococcal infections: role of protective humoral immunity. Infect Immun 1999; 67: 1871-7.

[58] Dong H**,** Xu G, Li S, *et al*. β-haemolytic group A streptococci *emm*75 carrying altered pyrogenic exotoxin A linked to scarlet fever in adults. J Infect 2008; 56: 261-7.

[59] Tyler SD, Johnson WM, Huang JC, *et al*. Streptococcal erythrogenic toxin genes: detection by polymerase chain reaction and association with disease in strains isolated in Canada from 1940-1991. J Clin Microbiol 1992; 30: 3127-31.

[60] Fraser JD, Proft T. The bacterial superantigen and superantigen-like proteins. Immunol Rev 2008; 225: 226-43.

[61] Blackman MA, Woodland DL. *In vivo* effects of superantigens. Life Sci 1995; 57: 1717-35.

[62] Li H, Llera A, Malchiodi EL, Mariuzza RA. The structural basis of T cell activation by superantigens. Ann Rev Immunol 1999; 17: 435-66.

[63] Ribeiro-Dias F, Shio MT, Timenetsky J, *et al*. *Mycoplasma arthritidis* superantigen (MAM)-induced macrophage nitric oxide release is MHC class II restricted, interferon gamma dependent, and toll-like receptor 4 independent. Exp Cell Res 2003; 286: 345-54.

[64] Ohmen JD, Modlin RL. Evidence for a superantigen in the pathogenesis of tuberculosis. Springer Semin Immunopathol 1996; 17: 375-84.

[65] Legaard PK, LeGrand RD, Misfeldt ML. The superantigen *Pseudomonas* exotoxin A requires additional functions from accessory cells for T lymphocyte proliferation. Cell Immunol 1991; 135: 372-82.

[66] Proft T, Moffat SL, Berkahn CJ, Fraser JD. Identification and characterization of novel superantigens from *Streptococcus pyogenes*. J Exp Med 1999; 189: 89-102.

[67] Stuart PM, Munn RK, DeMoll E, Woodward JG. Characterization of human T-cell responses to *Yersinia enterocolitica* superantigen. Hum Immunol 1995; 43: 269-75.

[68] Abe J, Kano H, Nogami H, *et al*. Pathogenic role of a superantigen in *Yersinia pseudotuberculosis* infection. Adv Exp Med Biol 2003; 529: 459-61.

[69] Malfitano AM, Cahill R, Mitchell P, *et al*. *Helicobacter pylori* has stimulatory effects on naïve T cells. Helicobacter 2006; 11: 21-30.

[70] Dobrescu D, Ursea B, Pope M, Asch AS, Posnett DN. Enhanced HIV-1 replication in V beta 12 T cells due to human cytomegalovirus in monocytes: evidence for a putative herpesvirus superantigen. Cell 1995; 82: 753-63.

[71] Hsiao FC, Lin M, Tai, A, Chen G, Huber BT. Cutting edge: Epstein-Barr virus transactivates the HERV-K18 superantigen by docking to the human complement receptor 2 (CD21) on primary B cells. J Immunol 2006; 177: 2056-60.

[72] Huber BT, Hsu PN, Sutkowski N. Virus-encoded superantigens. Microbiol Rev 1996; 60: 473-82.

[73] Acha-Orbea H, MacDonald HR. Superantigens of mouse mammary tumor virus. Ann Rev Immun 1995; 13: 459-86.

[74] Lafon M. Rabies virus superantigen. Res Immunol 1993; 144: 209-13.

[75] Devore-Carter D, Kar S, Vellucci V, Bhattacherjee V, Domanski P, Hostetter MK. Superantigen-like effects of a *Candida albicans* polypeptide. J Infect Dis 2008; 197: 981-9.

[76] Betley MJ, Borst DW, Regassa LB. Staphylococcal enterotoxins, toxic shock syndrome toxin and streptococcal pyrogenic exotoxins: a comparative study of their molecular biology. Chem Immunol 1992; 55: 1-35.

[77] Grumann D, Scharf SS, Holtfreter S, *et al*. Immune cell activation by enterotoxin gene cluster (*egc*)-encoded and non-*egc* superantigens from *Staphylococcus aureus*. J Immunol 2008; 181: 5054-61.

[78] Holtfreter S, Bauer K, Thomas D, *et al*. *egc*-Encoded superantigens from *Staphylococcus aureus* are neutralized by human sera much less efficiently than are classical staphylococcal enterotoxins or toxic shock syndrome toxin. Infect Immun 2004; 72: 4061-71.

[79] Vojtek I, Pirzada ZA, Henriques-Mormark B, Mastny M, Janapatla RP, Charpentier E. Lysogenic transfer of group A *Streptococcus* superantigen gene among streptococci. J Infect Dis 2008; 197: 225-34.

[80] Podbielski A, Woischnik M, Leonard BA, Schmidt KH. Characterization of *nra*, a global negative regulator gene in group A streptococci. Mol Microbiol 1999; 31: 1051-64.

[81] Broudy TB, Pancholi V, Fischetti VA. Induction of lysogenic bacteriophage and phage-associated toxin from group A streptococci during coculture with human pharyngeal cells. Infect Immun 2001; 69: 1440-3.

[82] Singh BR, Fen-Ni F, Ledoux DN. Crystal and solution structures of superantigenic staphylococcal enterotoxins compared. Struct Biol 1994; 1: 358-60.

[83] Papageorgiou AC, Tranter HS, Acharya KR. Crystal structure of microbial superantigen staphylococcal enterotoxin B at 1.5 angstrom resolution: implications for superantigen recognition by MHC class II molecules and T cell receptors. J Mol Biol 1998; 277: 61-79.

[84] Papageorgiou AC, Brehm RD, Deonidas DD, Tranter HS, Acharya KR. The refined crystal structure of toxic shock syndrome toxin-1 at 2.07 angstrom resolution. J Mol Biol 1996; 260: 553-69.

[85] Papageorgiou AC, Collins CM, Gutman DM, *et al*. Structural basis for the recognition of superantigen streptococcal pyrogenic exotoxin A (SpeA1) by MHC class II molecules and T-cell receptors. EMBO J 1999; 18: 9-21.

[86] Arcus V. OB-fold domains: a snapshot of the evolution of sequence, structure and function. Curr Opin Struct Biol 2002; 12: 794-801.

[87] Burroughs AM, Balaji S, Iyer LM, Aravind L. Small but versatile: the extraordinary functional and structural diversity of the beta-grasp fold. Biol Direct 2007; 2: 18.

[88] Arcus VL, Proft T, Sigrell JA, Baker HM, Fraser JD, Baker EN. Conservation and variation in superantigen structure and activity highlighted by the three-dimensional structures of two new superantigens from *Streptococcus pyogenes*. J Mol Biol 2000; 299: 157-68.

[89] Hurley JM, Shimonkevitz R, Hanagan A, *et al*. Identification of class II major histocompatibility complex and T cell receptor binding sites in the superantigen toxic shock syndrome toxin 1. J Exp Med 1995; 181: 2229-35.

[90] Kappler JW, Herman A, Clements J, Marrack P. Mutations defining functional regions of the superantigen staphylococcal enterotoxin B. J Exp Med 1992; 175: 387-96.

[91] Ulrich RG. Evolving superantigens of *Staphylococcus aureus*. FEMS Immunol Med Microbiol 2000; 27: 1-7.

[92] Kum WW, Chow AW. Inhibition of staphylococcal enterotoxin A-induced superantigenic and lethal activities by a monoclonal antibody to toxic shock syndrome toxin-1. J Infect Dis 2001; 183: 1739-48.

[93] Bavari S, Ulrich RG, LeClaire RD. Cross-reactive antibodies prevent the lethal effects of *Staphylococcus aureus* superantigens. J Infect Dis 1999; 180: 1365-9.

[94] Thompson NE, Ketterhagen MJ, Bergdoll MS. Monoclonal antibodies to staphylococcal enterotoxin B and C: cross-reactivity and localization of epitopes on tryptic fragments. Infect Immun 1984; 45: 281-5.

[95] Spero L, Morlock BA, Metzger JF. On the cross-reactivity of staphylococcal enterotoxins A, B, and C. J Immunol 1978; 120: 86-9.

[96] Bohach GA, Hovde CJ, Handley JP, Schlievert PM. Cross-neutralization of staphylococcal and streptococcal pyrogenic toxins by monoclonal and polyclonal antibodies. Infect Immun 1988; 56: 400-4.

[97] Chintagumpala MM, Mollick JA, Rich RR. Staphylococcal toxins bind to different sites on HLA-DR. J Immunol 1991; 147: 3876-81.

[98] Hudson KR, Tiedemann RE, Urban RG, Lowe SC, Strominger JL, Fraser JD. Staphylococcal enterotoxin A has two cooperative binding sites on major histocompatibility complex class II. J Exp Med 1995; 182: 711-20.

[99] Thibodeau J, Dohlsten M, Cloutier I, *et al*. Molecular characterization and role in T cell activation of staphylococcal enterotoxin A binding to the HLA-DR alpha-chain. J Immunol 1997; 158: 3698-704.

[100] Mehindate K, Thibodeau J, Dohlsten M, Kalland T, Sekaly RP, Mourad W. Cross-linking of major histocompatibility complex class II molecules by staphylococcal enterotoxin A superantigen is a requirement for inflammatory cytokine gene expression. J Exp Med 1995; 182: 1573-7.

[101] Jardetzky TS, Brown JH, Gorga JC, *et al*. Three-dimensional structure of a human class II histocompatibility molecule complexed with superantigen. Nature 1994; 368: 711-8.

[102] Kim J, Urban RG, Strominger JL, Wiley DC. Toxic shock syndrome toxin-1 complexed with a class II major histocompatibility molecule HLA-DR1. Science 1994; 266: 1870-4.

[103] Al-Daccak R, Mehindate K, Damdoumi F, *et al*. Staphylococcal enterotoxin D is a promiscuous superantigen offering multiple modes of interactions with the MHC class II receptors. J Immunol 1998; 160: 225-32.

[104] Kum WW, Wood JA, Chow AW. A mutation at glycine residue 31 of toxic shock syndrome toxin-1 defines a functional site critical for major histocompatiblity complex class II binding and superantigenic activity. J Infect Dis 1996; 174: 1261-70.

[105] Swietnicki W, Barnie AM, Dyas BK, Ulrich RG. Zinc binding and dimerization of *Streptococcus pyogenes* pyrogenic exotoxin C are not essential for T-cell stimulation. J Biol Chem 2003; 278: 9885-95.

[106] Seth A, Stern LJ, Ottenhoff TH, *et al*. Binary and ternary complexes between T-cell receptor, class II MHC and superantigen *in vitro*. Nature 1994; 369: 324-7.

[107] Ferry T, Thomas D, Perpoint T, *et al.* Analysis of superantigenic toxin V-beta T cell signatures produced during cases of staphylococcal toxic shock syndrome and septic shock. Clin Microbiol Infect 2008; 14: 546-54.

[108] Thomas D, Perpoint T, Dauwalder O, *et al. In vivo* and *in vitro* detection of a superantigenic toxin Vbeta signature in two forms of streptococcal toxic shock syndrome. Eur J Clin Microbiol Infect Dis 2009; 28: 671-6.

[109] Fields BA, Malchiodi EL, Li H, *et al.* Crystal structure of a T-cell receptor beta-chain complexed with a superantigen. Nature 1996; 384: 188-92.

[110] Li H, Llera A, Tsuchiya D, *et al.* Three-dimensional structure of the complex between a T cell receptor beta chain and the superantigen staphylococcal enterotoxin B. Immunity 1998; 9: 807-16.

[111] Petersson K, Pettersson H, Skartved NJ, Walse B, Forsberg G. Staphylococcal enterotoxin H induces V-alpha specific expansion of T cells. J Immunol 2003; 170: 4148-54.

[112] Nilsson H, Bjork P, Dohlsten M, Antonsson P. Staphylococcal enterotoxin H displays unique MHC class II binding properties. J Immunol 1999; 163: 6686-93.

[113] Lappin E, Ferguson AJ. Gram-positive toxic shock syndromes. Lancet Infect Dis 2009; 9: 281-90.

[114] Krakauer T. Induction of CC chemokines in human peripheral blood mononuclear cells by staphylococcal exotoxins and its prevention by pentoxifylline. J Leuk Biol 1999; 66: 158-64.

[115] Thomas D, Dauwalder O, Brun V, *et al. Staphylococcus aureus* superantigens elicit redundant and extensive human Vβ patterns. Infect Immun 2009; 77: 2043-50.

[116] Krakauer T, Stiles BG. Pentoxifylline inhibits superantigen-induced toxic shock and cytokine release. Clin Diagn Lab Immunol 1999; 6: 594-8.

[117] Hale ML, Margolin SB, Krakauer T, Roy CJ, Stiles BG. Pirfenidone blocks the *in vitro* and *in vivo* effects of staphylococcal enterotoxin B. Infect Immun 2002; 70: 2989-94.

[118] Muller-Alouf H, Proft T, Zollner TM, *et al*. Pyrogenicity and cytokine-inducing properties of *Streptococcus pyogenes* superantigens: comparative study of streptococcal mitogenic exotoxin Z and pyrogenic exotoxin A. Infect Immun 2001; 69: 4141-5.

[119] Hermann C, von Aulock S, Graf K, Hartung T. A model of human whole blood lymphokine release for *in vitro* and *ex vivo* use. J Immunol Meth 2003; 275: 69-79.

[120] Carlsson R, Fischer H, Sjogren HO. Binding of staphylococcal enterotoxin A to accessory cells is a requirement for its ability to activate human T cells. J Immunol 1988; 140: 2484-8.

[121] Fischer H, Dohlsten M, Andersson U, *et al.* Production of TNF-alpha and TNF-beta by staphylococcal enterotoxin A activated human T cells. J Immunol 1990; 144: 4663-9.

[122] Lagoo A, Lagoo-Deenadayalan S, Lorenz HM, Byrne J, Barber WH, Hardy KJ. IL-2, IL-4, and IFN-gamma gene expression versus secretion in superantigen-activated T cells. Distinct requirement for costimulatory signals through adhesion molecules. J Immunol 1994; 152: 1641-52.

[123] Trede NS, Geha RS, Chatila T. Transcriptional activation of IL-1 beta and tumor necrosis factor-alpha genes by MHC class II ligands. J Immunol 1991; 146: 2310-5.

[124] Lando P, Olsson C, Kalland T, Newton D, Kotb M, Dohlsten M. Regulation of superantigen-induced T cell activation in the absence and the presence of MHC class II. J Immunol 1996; 157: 2857-63.

[125] Hewitt CR, Lamb JR, Hayball J, Hill M, Owen MJ, O'Hehir RE. Major histocompatibility complex independent clonal T cell anergy by direct interaction of *Staphylococcus aureus* enterotoxin B with the T cell antigen receptor. J Exp Med 1992; 175: 1493-9.

[126] Stohl W, Elliott JE, Linsley PS. Human T cell-dependent B cell differentiation induced by staphylococcal superantigens. J Immunol 1994; 153: 117-27.

[127] Van Zele T, Gevaert P, Holtappels G, van Cauwenberge P, Bachert C. Local immunoglobulin production in nasal polyposis is modulated by superantigens. Clin Exp Allergy 2007; 37: 1840-7.

[128] Lonnqvist A, Ostman S, Almqvist N, *et al.* Neonatal exposure to staphylococcal superantigen improves induction of oral tolerance in a mouse model of airway allergy. Eur J Immunol 2009; 39: 447-56.

[129] Tran CN, Lundy SK, White PT, *et al.* Molecular interactions between T cells and fibroblast-like synoviocytes: role of membrane tumor necrosis factor-alpha on cytokine-activated T cells. Am J Pathol 2007; 171: 1588-98.

[130] Wooley PH, Cingel B. Staphylococcal enterotoxin B increases the severity of type II collagen induced arthritis in mice. Ann Rheum Dis 1995; 54: 298-304.

[131] Kraft M, Filsinger S, Kramer KL, Kabelitz D, Hansch GM, Schoels M. Synovial fibroblasts as accessory cells for staphylococcal enterotoxin-mediated T-cell activation. Immunology 1995; 85: 461-5.

[132] Pinchuk IV, Beswick EJ, Saada JI, *et al.* Monocyte chemoattractant protein-1 production by intestinal myofibroblasts in response to staphylococcal enterotoxin A: relevance to staphylococcal enterotoxigenic disease. J Immunol 2007; 178: 8097-106.

[133] Scheuber PH, Denzlinger C, Wilker D, Beck G, Keppler D, Hammer DK. Staphylococcal enterotoxin B as a nonimmunological mast cell stimulus in primates: the role of endogenous cysteinyl leukotrienes. Int Arch Allergy Appl Immunol 1987; 82: 289-91.

[134] Hu DL, Zhu G, Mori F, *et al.* Staphylococcal enterotoxin induces emesis through increasing serotonin release in intestine and it is downregulated by cannabinoid receptor 1. Cell Microbiol 2007; 9: 2267-77.

[135] Raj HD, Bergdoll MS. Effect of enterotoxin B on human volunteers. J Bacteriol 1969; 98: 833-4.

[136] Schlievert PM, Jablonski LM, Roggiani M, *et al.* Pyrogenic toxin superantigen site specificity in toxic shock syndrome and food poisoning in animals. Infect Immun 2000; 68: 3630-4.

[137] Spiekermann GM, Nagler-Anderson C. Oral administration of the bacterial superantigen staphylococcal enterotoxin B induces

activation and cytokine production by T cells in murine gut-associated lymphoid tissue. J Immunol 1998; 161: 5825-31.

[138] Lu J, Wang A, Ansari S, Hershberg RM, McKay DM. Colonic bacterial superantigens can evoke an inflammatory response and exaggerate disease in mice recovering from colitis. Gastroenterology 2003; 125: 1785-95.

[139] Sugiyama H, Bergdoll MS, Dack GM. Early development of a temporary resistance to the emetic action of staphylococcal enterotoxin. J Infect Dis 1962; 111: 233-8.

[140] Hamad AR, Marrack P, Kappler JW. Transcytosis of staphylococcal superantigen toxins. J Exp Med 1997; 185: 1447-54.

[141] Buxser S, Bonventre PF. Staphylococcal enterotoxins fail to disrupt membrane integrity or synthetic functions of Henle 407 intestinal cells. Infect Immun 1981; 31: 929-34.

[142] Lu J, Philpott DJ, Saunders PR, Perdue MH, Yang PC, McKay DM. Epithelial ion transport and barrier abnormalities evoked by superantigen-activated immune cells are inhibited by interleukin-10 but not interleukin-4. J Pharm Exper Ther 1998; 287: 128-36.

[143] Harris TO, Grosmann D, Kappler JR, Marrack P, Rich RR, Betley MJ. Lack of complete correlation between emetic and T-cell-stimulatory activities of staphylococcal enterotoxins. Infect Immun 1993; 61: 3175-83.

[144] Stelma GN, Bergdoll MS. Inactivation of staphylococcal enterotoxin A by chemical modification. Biochem Biophys Res Commun 1982; 105: 121-6.

[145] Alber G, Hammer DK, Fleischer B. Relationship between enterotoxic- and T lymphocyte-stimulating activity of staphylococcal enterotoxin B. J Immunol 1990; 144: 4501-6.

[146] Reck B, Scheuber PH, Londong W, Sailer-Kramer B, Bartsch K, Hammer DK. Protection against the staphylococcal enterotoxin-induced intestinal disorder in the monkey by anti-idiotypic antibodies. Proc Natl Acad Sci USA 1988; 85: 3170-4.

[147] Hoffman M, Tremaine M, Mansfield J, Betley M. Biochemical and mutational analysis of the histidine residues of staphylococcal enterotoxin A. Infect Immun 1996; 64: 885-90.

[148] McCormack JE, Callahan JE, Kappler J, Marrack PC. Profound deletion of mature T cells *in vivo* by chronic exposure to exogenous superantigen. J Immunol 1993; 150: 3785-92.

[149] Miethke T, Wahl C, Gaus H, Heeg K, Wagoner H. Exogenous superantigens acutely trigger distinct levels of peripheral T cell tolerance/immunosuppression: dose-response relationship. Eur J Immunol 1994; 24: 1893-902.

[150] Saloga J, Enk AH, Becker D, Bellinghausen I, Kuhn S, Knop J. Active suppression induced by cutaneous exposure to bacterial superantigen is prevented by interleukin-12 treatment *in vivo*. Immunology 1998; 93: 485-92.

[151] Feunou P, Vanwetswinkel S, Gaudray F, Goldman M, Matthys P, Braun MY. $Foxp^{3+}CD25^{+}$ T regulatory cells stimulate IFN-gamma-independent CD152-mediated activation of tryptophan catabolism that provides dendritic cells with immune regulatory activity in mice unresponsive to staphylococcal enterotoxin B. J Immunol 2007; 179: 910-7.

[152] Collins LV, Eriksson K, Ulrich RG, Tarkowski A. Mucosal tolerance to a bacterial superantigen indicates a novel pathway to prevent toxic shock. Infect Immun 2002; 70: 2282-7.

[153] Stiles BG, Campbell YG, Castle RM, Grove SA. Correlation of temperature and toxicity in murine studies of staphylococcal enterotoxins and toxic shock syndrome toxin 1. Infect Immun 1999; 67: 1521-5.

[154] Bean AG, Freiburg RA, Andrade S, Menon S, Zlotnik A. Interleukin 10 protects mice against staphylococcal enterotoxin B-induced lethal shock. Infect Immun 1993; 61: 4937-9.

[155] Bhatnagar A, Grover A, Ganguly NK. Superantigen-induced T cell responses in acute rheumatic fever and chronic rheumatic heart disease patients. Clin Exp Immunol 1999; 116: 100-6.

[156] Miethke T, Wahl C, Heeg K, Echentacher T, Krammer PH, Wagner H. T cell-mediated lethal shock triggered in mice by the superantigen staphylococcal enterotoxin B: critical role of tumor necrosis factor. J Exp Med 1992; 175: 91-8.

[157] Schlievert PM. Role of superantigens in human disease. J Infect Dis 1993; 167: 997-1002.

[158] McCormick JK, Pragman AA, Stolpa JC, Leung DY, Schlievert PM. Functional characterization of streptococcal pyrogenic exotoxin J, a novel superantigen. Infect Immun 2001; 69: 1381-8.

[159] Okusawa S, Gelfand JA, Ikejima T, Connolly RJ, Dinarello CA. Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J Clin Invest 1988; 81: 1162-72.

[160] Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. J Endotoxin Res 2004; 10: 201-22.

[161] Sugiyama H, McKissic EM, Bergdoll MS, Heller B. Enhancement of bacterial endotoxin lethality by staphylococcal enterotoxin. J Infect Dis 1964; 114: 111-8.

[162] Stiles BG, Bavari S, Krakauer T, Ulrich RG. Toxicity of staphylococcal enterotoxins potentiated by lipopolysaccharide: major histocompatibility complex class II molecule dependency and cytokine release. Infect Immun 1993; 61: 5333-8.

[163] Chen JY, Qiao Y, Komisar JL, Baze WB, Hsu IC, Tseng J. Increased susceptibility to staphylococcal enterotoxin B intoxication in mice primed with actinomycin D. Infect Immun 1994; 62: 4626-31.

[164] Sarawar SR, Blackman MA, Doherty PC. Superantigen shock in mice with an inapparent viral infection. J Infect Dis 1994; 170: 1189-94.

[165] Dinges MM, Schlievert PM. Comparative analysis of lipopolysaccharide-induced tumor necrosis factor alpha activity in serum and lethality in mice and rabbits pretreated with the staphylococcal superantigen toxic shock syndrome toxin 1. Infect Immun 2001; 69: 7169-72.

[166] Huzella LM, Buckley MJ, Alves DA, Stiles BG, Krakauer T. Central roles for IL-2 and MCP-1 following intranasal exposure to SEB: a new mouse model. Res Vet Sci 2009; 86: 241-7.

[167] Dinges MM, Schlievert PM. Role of T cells and gamma interferon during induction of hypersensitivity to lipopolysaccharide by toxic shock syndrome toxin 1 in mice. Infect Immun 2001; 69: 1256-64.

[168] Paiva CN, Pyrrho AS, Lannes-Vieira J, Vacchio M, Soares MB, Gattass CR. *Trypanosoma cruzi* sensitizes mice to fulminant SEB-induced shock: over release of inflammatory cytokines and independence of Chagas' disease or TCR Vβ-usage. Shock 2003; 19: 163-8.

[169] Stone RL, Schlievert PM. Evidence for the involvement of endotoxin in toxic shock syndrome. J Infect Dis 1987; 155: 682-9.

[170] Beno DW, Uhing MR, Goto M, Chen Y, Jiampaya-Serna VA, Kimura RE. Chronic staphylococcal enterotoxin B and lipopolysaccharide induce a bimodal pattern of hepatic dysfunction and injury. Crit Care Med 2003; 31: 1154-9.

[171] LeClaire RD, Hunt RE, Bavari S, Estep JE, Nelson GO, Wilhelmsen CL. Potentiation of inhaled staphylococcal enterotoxin B-induced toxicity by lipopolysaccharide in mice. Toxicol Pathol 1996; 24: 619-26.

[172] Schlievert PM. Enhancement of host susceptibility to lethal endotoxin shock by staphylococcal pyrogenic exotoxin type C. Infect Immun 1982; 36: 123-8.

[173] Holtfreter S, Broker BM. Staphylococcal superantigens: do they play a role in sepsis? Arch Immunol Ther Exp 2005; 53: 13-27.

[174] Draisma A, Pickkers P, Bouw MP, van der Hoeven JG. Development of endotoxin tolerance in humans *in vivo*. Crit Care Med 2009; 37: 1261-7.

[175] Chow AW, Bartlett KH, Percival-Smith R, Morrison BJ. Vaginal colonization with *Staphylococcus aureus*, positive for toxic-shock marker protein, and *Escherichia coli* in healthy women. J Infect Dis 1984; 150: 80-4.

[176] Hasko G, Virag L, Egnaczyk G, Salzman AL, Szabo C. The crucial role of IL-10 in the suppression of the immunological response in mice exposed to staphylococcal enterotoxin B. Eur J Immunol 1998; 28: 1417-25.

[177] Krakauer T. Immune response to staphylococcal superantigens. Immunol Res 1999; 20: 163-73.

[178] Saha B, Harlan DM, Lee KP, June CH, Abe R. Protection against lethal toxic shock by targeted disruption of the CD28 gene. J Exp Med 1996; 183: 2675-80.

[179] Mittrucker HW, Shahinan A, Bouchard D, Kundig TM, Mak TW. Induction of unresponsiveness and impaired T cell expansion by staphylococcal enterotoxin B in CD28-deficient mice. J Exp Med 1996; 183: 2481-8.

[180] Xu H, Gonzalo JA, St. Pierre Y, *et al*. Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. J Exp Med 1994; 180: 95-109.

[181] Manjunath N, Correa M, Ardman M, Ardman B. Negative regulation of T-cell adhesion and activation by CD43. Nature 1995; 377: 535-8.

[182] Mountz JD, Baker TJ, Borcherding DR, Bluethmann H, Zhou T, Edwards CK. Increased susceptibility of fas mutant MRL-lpr/lpr mice to staphylococcal enterotoxin B-induced septic shock. J Immunol 1995; 155: 4829-37.

[183] Yeung RS, Penninger JM, Kundig T, *et al*. Human CD4 and human major histocompatibility complex class II (DQ6) transgenic mice: supersensitivity to superantigen-induced septic shock. Eur J Immun 1996; 26: 1074-82.

[184] DaSilva L, Welcher BC, Ulrich RG, Aman MJ, David CS, Bavari S. Human-like immune responses of human leukocyte antigen-DR3 transgenic mice to staphylococcal enterotoxins: a novel model for superantigen vaccines. J Infect Dis 2002; 185: 1754-60.

[185] Welcher BC, Carra JH, DaSilva L, *et al*. Lethal shock induced by streptococcal pyrogenic exotoxin A in mice transgenic for human leukocyte antigen-DQ8 and human CD4 receptors: implications for development of vaccines and therapeutics. J Infect Dis 2002; 186: 501-10.

[186] Zhao Y-X, Abdelnour A, Kalland T, Tarkowski A. Overexpression of the T-cell receptor Vbeta 3 in transgenic mice increases mortality during infection by enterotoxin A-producing *Staphylococcus aureus*. Infect Immun 1995; 63: 4463-9.

[187] Vlach KD, Boles JW, Stiles BG. Telemetric evaluation of body temperature and physical activity as predictors of mortality in a murine model of staphylococcal enterotoxic shock. Comp Med 2000; 50: 160-6.

[188] Boles JW, Pitt ML, LeClaire RD, Gibbs PH, Ulrich RG, Bavari S. Correlation of body temperature with protection against staphylococcal enterotoxin B exposure and use in determining vaccine dose-schedule. Vaccine 2003; 21: 2791-6.

[189] Hamel M, Eynon EE, Savelkoul HF, van Oudenaren A, Kruisbeek AM. Activation and re-activation potential of T cells responding to staphylococcal enterotoxin B. Int Immunol 1995; 7: 1065-77.

[190] Ivars F. Superantigen-induced regulatory T cells *in vivo*. Chem Immunol Allergy 2007; 93: 137-60.

[191] MacDonald HR, Lees RK, Baschieri S, Herrmann T, Lussow AR. Peripheral T-cell reactivity to bacterial superantigens *in vivo*: the response/anergy paradox. Immunol Rev 1993; 133: 105-17.

[192] Florquin S, Amraoui Z, Goldman M. T cells made deficient in interleukin-2 production by exposure to staphylococcal enterotoxin B *in vivo* are primed for interferon-gamma and interleukin-10 secretion. Eur J Immunol 1995; 25: 1148-53.

[193] Parsonnet J, Gillis ZA, Richter AG, Pier GB. A rabbit model of toxic shock syndrome that uses a constant, subcutaneous infusion of toxic shock syndrome toxin 1. Infect Immun 1987; 55: 1070-6.

[194] Huang WT, Lin MT, Won SJ. Staphylococcal enterotoxin A-induced fever is associated with increased circulating levels of cytokines in rabbits. Infect Immun 1997; 65: 2656-62.

[195] Lee PK, Schlievert PM. Quantification and toxicity of group A streptococcal pyrogenic exotoxins in an animal model of toxic shock syndrome-like illness. J Clin Microbiol 1989; 27: 1890-2.

[196] De Azavedo JCS, Arbuthnott JP. Toxicity of staphylococcal toxic shock syndrome toxin 1 in rabbits. Infect Immun 1984; 46: 314-7.

[197] Pettit GW, Elwell MR, Jahrling PB. Possible endotoxemia in rabbits after intravenous injection of *Staphylococcus aureus* enterotoxin B. J Infect Dis 1977; 135: 646-8.

[198] Fujiwaka H, Igarashi H, Usami H, Tanaka S, Tamura H. Clearance of endotoxin from blood of rabbits injected with staphylococcal toxic shock syndrome toxin-1. Infect Immun 1986; 52: 134-7.

[199] Klintman D, Li X, Sato T, Wang Y, Jeppson B, Thoriacius H. Staphylococcal enterotoxin A-induced hepatotoxicity is predominantly mediated by Fas ligand (CD95L). Ann Surg 2004; 240: 1065-72.

[200] Van Miert A, Van Duin C, Schotman A. Comparative observations of fever and associated clinical hematological and blood biochemical changes after intravenous administration of staphylococcal enterotoxins B and F (toxic shock syndrome toxin-1) in goats. Infect Immun 1984; 46: 354-60.

[201] Wright A, Andrews P, Titball RW. Induction of emetic, pyrexic, and behavioral effects of *Staphylococcus aureus* enterotoxin B in the ferret. Infect Immun 2000; 68: 2386-9.

[202] Hu D-L, Omoe K, Shimoda Y, Nakane A, Shinagawa K. Induction of emetic response to staphylococcal enterotoxins in the house musk shrew (*Suncus murinus*). Infect Immun 2003; 71: 567-70.

[203] Hu D-L, Omoe K, Sashinami H, Shinagawa K, Nakane A. Immunization with a nontoxic mutant of staphylococcal enterotoxin A, SEAD227A, protects against enterotoxin-induced emesis in house musk shrews. J Infect Dis 2009; 199: 302-10.

[204] Desouza IA, Franco-Penteado CF, Camargo EA, *et al*. Inflammatory mechanisms underlying the rat pulmonary neutrophil influx induced by airway exposure to staphylococcal enterotoxin type A. Br J Pharmacol 2005; 146: 781-91.

[205] Won S-J, Huang WT, Lai YS, Lin MT. Staphylococcal enterotoxin A acts through nitric oxide synthase mechanisms in human peripheral blood mononuclear cells to stimulate synthesis of pyrogenic cytokines. Infect Immun 2000; 68: 2003-8.

[206] Saha B, Jaklic B, Harlan DM, Gray GS, June CH, Abe R. Toxic shock syndrome toxin-1 induced death is prevented by CTLA4Ig. J Immunol 1996; 157: 3869-75.

[207] Takei Y, Kunikata T, Aga M, *et al*. Tryptanthrin inhibits interferon-γ production by Peyer's patch lymphocytes derived from mice that had been orally administered staphylococcal enterotoxin. Biol Pharm Bull 2003; 26: 365-7.

[208] Miller EJ, Cohen AB, Peterson BT. Peptide inhibitor of interleukin-8 (IL-8) reduces staphylococcal enterotoxin-A (SEA) induced neutrophil trafficking to the lung. Inflamm Res 1996; 45: 393-7.

[209] Buonpane RA, Churchill HRO, Moza B, *et al*. Neutralization of staphylococcal enterotoxin B by soluble, high-affinity receptor antagonists. Nat Med 2007; 13: 725-9.

[210] Eriksson BK, Andersson J, Holm SE, Norgren M. Invasive group A streptococcal infections: T1M1 isolates expressing pyrogenic exotoxins A and B in combination with selective lack of toxin-neutralizing antibodies are associated with increased risk of streptococcal toxic shock syndrome. J Infect Dis 1999; 180: 410-8.

[211] Norrby-Teglund A, Kaul R, Low DE, *et al*. Plasma from patients with severe invasive group A streptococcal infections treated with normal polyspecific IgG inhibits streptococcal superantigen-induced T cell proliferation and cytokine production. J Immunol 1996; 156: 3057-64.

[212] Schlievert PM. Use of intravenous immunoglobulin in the treatment of staphylococcal and streptococcal toxic shock syndromes and related illnesses. J Allergy Clin Immunol 2001; 108: S107-10.

[213] LeClaire RD, Hunt RE, Bavari S. Protection against bacterial superantigen staphylococcal enterotoxin B by passive vaccination. Infect Immun 2002; 70: 2278-81.

[214] Woody MA, Krakauer T, Stiles BG. Staphylococcal enterotoxin B mutants (N23K and F44S): biological effects and vaccine potential in a mouse model. Vaccine 1997; 15: 133-9.

[215] Woody MA, Krakauer T, Ulrich RG, Stiles BG. Differential immune responses to staphylococcal enterotoxin B mutations in a hydrophobic loop dominating the interface with major histocompatibility complex class II receptors. J Infect Dis 1998; 177: 1013-22.

[216] Savransky V, Pinelis D, Korolev S, Ionin B, Fegeding K. Immunogenicity of the histidine-to-tyrosine staphylococcal enterotoxin B mutant protein in C3H/HeJ mice. Toxicon 2004; 43: 433-8.

[217] Boles JW, Pitt ML, LeClaire RD, *et al*. Generation of protective immunity by inactivated recombinant staphylococcal enterotoxin B vaccine in nonhuman primates and identification of correlates of immunity. Clin Immunol 2003; 108: 51-9.

[218] Narita K, Hu DL, Tsuji T, Nakane A. Intranasal immunization of mutant toxic shock syndrome toxin 1 elicts systemic and mucosal immune response against *Staphylococcus aureus* infection. FEMS Immunol Med Microbiol 2008; 52: 389-96.

[219] Bergdoll MS. Immunization of rhesus monkeys with enterotoxoid B. J Infect Dis 1966; 116: 191-6.

[220] Lowell GH, Kaminski RW, Grate S, *et al*. Intranasal and intramuscular proteosome-staphylococcal enterotoxin B (SEB) toxoid vaccines: immunogenicity and efficacy against lethal SEB intoxication in mice. Infect Immun 1996; 64: 1706-13.

[221] di Tommaso A, de Magistris MT, Bugnoli M, Marsili I, Rappuoli R, Abrignani S. Formaldehyde treatment of proteins can constrain presentation to T cells by limiting antigen processing. Infect Immun 1994; 62: 1830-4.

[222] Cropley I, Douce G, Roberts M, *et al*. Mucosal and systemic immunogenicity of a recombinant, non-ADP-ribosylating pertussis toxin: effects of formaldehyde treatment. Vaccine 1995; 13: 1643-8.

[223] Rott O, Fleischer B. A superantigen as virulence factor in an acute bacterial infection. J Infect Dis 1994; 169: 1142-6.

[224] Edwin C, Tatini SR, Maheswaran SK. Nature and reactivity of staphylococcal enterotoxin A monoclonal antibodies. Appl Environ Microbiol 1986; 52: 1247-52.

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