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 a year 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...
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 vitro [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°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 (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.

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 (Kd = 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 Zn²⁺-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
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\textsuperscript{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\textsuperscript{2+} – dependent binding to MHC II; this is not essential for T-cell activation and suggests Zn\textsuperscript{2+} – independent interactions [105]. The evolutionary similarity between staphylococcal and streptococcal 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\textsuperscript{-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 T cell 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 chemotactic 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 Vβ\textsuperscript{+} 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 SAgs as a neonate, via colonization by toxigenic *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 I). 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 Vβ\textsuperscript{8} 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
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 (i.e. non-human primates or rabbits) for studying SAg-mediated shock (Table 1) [156, 161-166]. Relative to non-SEA, SEB + D galactosamine or SEB + Actinomycin D [163] increases transcytosis by fifty to seventy percent. In mice also elicit a dose-related tolerance within sixteen hours 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 effects, and skin reactivity [133, 146, 147]. In non-human primates, this modified SEB inhibits vomiting and diarrhea of 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.

SAs 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,
lipopoly saccharide (LPS), viruses (influenza and
lymphocytic choriomeningitis), or even pathogenic protozoa
(Trypansoma 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-, SE-, 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
(inter cellular 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 Vß3 (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
Vß-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 Vß-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 Vß8’ 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 Kₐ 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]. Recombinant-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.

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