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Staphylococcus aureus and Streptococcus pyogenes are facultative gram-positive cocci that play an important role in a myriad of human illnesses, including food poisoning, skin infections, pharyngitis, life threatening shock, and autoimmune disorders like arthritis, psoriasis, and atopic dermatitis. These ubiquitous bacteria produce various virulence factors that include superantigens like staphylococcal enterotoxins (SE), toxic shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic exotoxins (SPE). To date, the continually expanding literature describes 15 SE and 6 SPE that differ in amino acid sequence and biological effects (i.e. activation of specific T cells). Picomolar concentrations of these toxins activate specific VB-bearing T cells after binding to major histocompatibility complex class II molecules found on antigen presenting cells. The activated T cells vigorously proliferate with a concomitant production of various proinflammatory cytokines, which in sufficient quantities elicit fever, hypotension, and lethal shock. Various in vitro and in vivo models have been developed to study SE, TSST-1, and SPE, thus providing invaluable tools for understanding how these toxins work and discovering potential vaccines as well as therapeutics. This review summarizes the biological properties of, and potential neutralization strategies for, the SE, TSST-1, and SPE.

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1 Staphylococcal enterotoxins, toxic shock syndrome toxin-1, and streptococcal pyrogenic exotoxins: Some basic biology of bacterial superantigens

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Abstract

Staphylococcus aureus and Streptococcus pyogenes are facultative gram-positive cocci that play an important role in a myriad of human illnesses, including food poisoning, skin infections, pharyngitis, life threatening shock, and autoimmune disorders like arthritis, psoriasis, and atopic dermatitis. These ubiquitous bacteria produce various virulence factors that include superantigens like staphylococcal enterotoxins (SE), toxic shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic exotoxins (SPE). To date, the continually expanding literature describes 15 SE and 6 SPE that differ in amino acid sequence and biological effects (i.e. activation of specific T cells). Picomolar concentrations of these toxins activate specific Vβ-bearing T cells after binding to major histocompatibility complex class II

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molecules found on antigen presenting cells. The activated T cells vigorously proliferate with a concomitant production of various proinflammatory cytokines, which in sufficient quantities elicit fever, hypotension, and lethal shock. Various in vitro and in vivo models have been developed to study SE, TSST-1, and SPE, thus providing invaluable tools for understanding how these toxins work and discovering potential vaccines as well as therapeutics. This review summarizes the biological properties of, and potential neutralization strategies for, the SE, TSST-1, and SPE.

Introduction

Staphylococcus aureus and *Streptococcus pyogenes* represent ubiquitous and formidable pathogens associated with numerous human and animal diseases [1-3]. These facultative, β–hemolytic bacteria can readily colonize the skin or various mucosal surfaces and once established, they can produce numerous virulence factors that aid in their survival and subsequent dissemination. In addition to the single chain staphylococcal enterotoxins (SE) and toxic shock syndrome toxin 1 (TSST-1), which may down regulate an immune response of a colonized host by knocking out specific subsets of T cells [4-7], *S. aureus* can also produce protein A, coagulases, hemolysins, leukocidins, and inactivate complement [8,9]. A frightening reality is the increasing resistance of *S. aureus* to antibiotics like methicillin [10], and our current last line of defense which has recently been breached, vancomycin [11,12]. This sobering fact is further reinforced by data from Canada, where it is estimated that ~50 million dollars are spent each year for managing antibiotic resistant *S. aureus* in hospitals, and the cost to the dairy industry is even higher [13]. Indeed, *S. aureus* truly represents an important health and economic concern for all.

It was with serendipity, vision, and a fungal-contaminated petri plate originally seeded with *S. aureus* that Alexander Fleming brilliantly initiated the antibiotic era in 1927. This revolutionary means of microbial control was tested in humans 14 years later with a new "wonder drug", penicillin, and a patient dying from a staphylococcal infection [14]. The efforts of many scientists and physicians during, and after, World War II accelerated the discovery, and use, of various antimicrobials that we still rely heavily upon sixty years later to target *S. aureus* and other marauding microbes that are locked in a perpetual chess match for survival with their host. Clearly, an understanding of how a pathogen survives, flourishes, and evolves can go a long way towards developing alternative means of control. Part of this review delves into an important group of protein toxins produced by *S. aureus* that have intrigued bacteriologists and immunologists for decades: the SE and TSST-1.

The SE (serotypes A - P) are 25 - 30 kD, single chain proteins associated with one of the most prevalent forms of food poisoning in the United States and throughout the world [2,15]. The first report of human staphylococcal food poisoning was in 1914 following consumption of warm milk from a cow with *S. aureus*-induced mastitis [16]. SE poisoning typically occurs after ingesting processed meats or dairy products previously contaminated by improper handling and subsequently stored at elevated temperatures, thus resulting in *S. aureus* growth and production of one or more SE. Only microgram amounts of preformed toxin can elicit emesis and diarrhea within ~ 4 h after consumption, and one may still experience a general malaise 24 - 48 h later [16]. SE food poisoning is rarely fatal among reasonably healthy, noncompromised individuals.

How these toxins elicit enteric effects is still unresolved and equivocal, but inflammatory compounds such as prostaglandins and leukotrienes may play a role [17,18]. Of the various SE serotypes, A is most commonly associated with food poisoning [19]. It is evident that various populations throughout the world are naturally exposed to these toxins, as demonstrated by SEB seroconversion rates [20]. Whether humans develop toxin-specific antibodies following ingestion of contaminated food and/or colonization by a toxin-producing strain of *S. aureus* still remains a mystery.

In contrast with the food-borne SE, toxic shock syndrome (TSS) caused by *S. aureus* TSST-1 (22 kD, single-chain protein) was first described by Todd et al. in 1978 [21] and was later found intimately associated with menstruation and use of highly absorbent tampons [22-25]. Elevated levels of protein, carbon dioxide, or oxygen, a more neutral pH, and/or removal of Mg^{2+} ions from the microenvironment of vaginally located *S. aureus* have been implicated in increased growth and production of TSST-1 in vivo [15, 26-28]. TSST-1 was originally thought to be an enterotoxin, and thus some early literature describes it as SEF [29]. However, this later proved to be a misnomer as homogeneous TSST-1 lacks enterotoxicity in non-human primates [30]. The symptoms of TSST-1 intoxication, referred to as TSS and linked to an altered immune response that includes elevated serum levels of proinflammatory cytokines [31-33], consists of rash, hypotension, fever, and multisystem dysfunction [34,35]. Although less common, non-menstrual TSS in men, women, and children is also attributed to SEB and SEC1 production by *S. aureus* growing at other body sites [36-38]. Unlike the decreased number of menstrual TSS cases since the early 1980s, a likely result of heightened public awareness and reformulated tampons, non-menstrual cases of TSS have remained constant. All types of TSS patients may suffer recurring bouts unless the offending strain of *S. aureus* is eliminated or at least kept at a minimal growth level. Antibodies seem to play an important role in human susceptibility to TSST-1-induced TSS [37,39-41], thus individuals that do not seroconvert towards the toxin due to TSST-1-induced hyporesponsive T cells [42] and/or T-cell dependent B-cell apoptosis [43] are more likely to experience recurring bouts. Perhaps these findings further underscore the importance of experimental vaccines that may break tolerance towards TSST-1 and other bacterial superantigens, especially among populations at high risk [44-54].

A close cousin of *S. aureus* is *S. pyogenes*, which represents the group A streptococci as defined by the classic carbohydrate-based, serotyping system developed in the 1930s by Rebecca Lancefield [55]. The normal niches for *S. pyogenes*, like *S. aureus*, are the skin and mucosal surfaces of a host. Group A steptococci can cause a myriad of human diseases, including pharyngitis (strep throat), impetigo, necrotizing fasciitis, scarlet fever, and rheumatic fever [56]. Microscopically, it is relatively easy to distinguish *S. pyogenes* from *S. aureus* following a gram stain, as the former classically appears as chains and not as "grape-like clusters". Biochemically, *S. pyogenes* is easily discerned from *S. aureus*, as it is catalase negative. However, like *S. aureus*, *S. pyogenes* does possess a potent arsenal of virulence factors that includes antiphagocytic mechanisms such as a capsule, M protein, and protease that cleaves complement C5a [57]. Protein G of *S. pyogenes*, like protein A of *S. aureus*, readily binds to the Fc region of immunoglobulins (Ig) and subsequently interferes with antibody-mediated opsonization. *S. pyogenes* also produces hemolysins such as streptolysins O and S that are oxygen labile and stable, respectively. An ability by *S. pyogenes* to convert plasminogen into plasmin, which then digests fibrin clots, further enables this microbe to

disseminate from an infected body site. In addition to *S. aureus* SE and TSST-1, this review will also address the biology of streptococcal pyrogenic exotoxins (SPE) produced by *S. pyogenes*. Like SE and TSST-1, the SPE can also cause TSS and are superantigenic.

The term "superantigen" was first coined in the late 1980s by Marrack and Kappler [58,59] to describe microbial (bacterial or viral) proteins that activate a large proportion of specific T cells (5 - 30 % of total population) at picogram concentrations, which contrasts with most conventional antigens that normally stimulate < 0.01% of the T cells at higher concentrations [58-64]. There are a large number of microbial superantigens described in the literature (Table 1). To date, various gram-positive and gram-negative bacteria, as well as viruses, have been identified as producers of protein superantigens. Superantigens from mouse mammary tumor virus (MMTV) represent those best characterized of viral origin [65]. The superantigens from MMTV differ from bacterial sources in that they are endogenous and produced as membrane glycoproteins that require proteolytic processing before activating T cells [66-68]. Viral superantigenic-like activities for human T lymphocytes have also been reported for rabies [69], Epstein-Barr virus (EBV) [70], herpesvirus saimari [71], cytomegalovirus [72] and human immunodeficiency virus (HIV) [73], but only the nucleocapsid and nef proteins from rabies [74] and HIV [75,76] have respectively been isolated and identified as human viral superantigens. Recent studies suggest that the envelope genes of human endogenous retrovirus (HERV)-K18 encode a superantigen with specificity for $V\beta7^+$ and $V\beta13^+$ T cells [77-78]. EBV can transactivate the (HERV)-K18 genes and interferon alpha (IFNα) regulates expression of this viral superantigen [79].

Interactions of superantigens with host cells differ from those of conventional antigens, in that superantigens: 1) directly bind outside the peptide-binding groove of major histocompatibility complex (MHC) class II, 2) exert biological effects as an intact

Table 1. Bacterial and viral superantigens

Source	Superantigen
Bacterial	
Staphylococcus aureus	SEA-SEP, TSST-1
Streptococcus pyogenes	SPEA, C, G, H, I, and J, streptococcal mitogenic exotoxin z (SMEZ), streptococcal superantigen (SSA)
Yersinia enterocolitica	YES
Yersinia pseudotuberculosis	YPM
Pseudomonas aeruginosa	Exotoxin A
Mycoplasma arthritidis	MAM
Mycobacterium tuberculosis	MTS
Viral	
Mouse Mammary Tumor Virus	MMTV viral superantigens
Epstein-Barr Virus	Not determined
Rabies Virus	Nucleocapsid protein
Cytomegalovirus	Not determined
Human Immunodeficiency Virus	Nef
Herpes virus saimari	HVS 14 protein

molecule without "processing", and 3) are not MHC class II restricted but there are differences between alleles (i.e., human HLA-DR, -DQ, -DP or murine IA and IE) and how they present superantigens to T cells [58,59]. Furthermore, recognition of a superantigen by the T-cell receptor (TCR) is dependent upon the variable region of the β chain (Vβ) from the TCR, and not the combination of Vα and Vβ chains, as in the case of conventional peptide antigens [1,58,59,80]. Because of its ability to interact with MHC class II and TCR, a superantigen stimulates both antigen-presenting cells (APC) and T lymphocytes. This activation leads to massive production of cytokines and chemokines, enhances expression of cell dhesion molecules, and increases T-cell proliferation, apoptosis as well as anergy.

Physical characteristics

The SE, TSST-1, and SPE are 22- to 30-kD single chain proteins divided into common homology groups based upon primary sequence [58,81]. It is possible that in the past, *S. aureus* and *S. pyogenes* obtained common segments of DNA that ultimately yielded divergently evolved, yet closely related, superantigens; however, to date this has not been conclusively proven. These toxins are usually encoded by plasmids, bacteriophages, or mobile genetic elements and appear during the late logarithmic to stationary phase of growth in vitro [15,82]. Among the different SE "serotypes", SEA, SED, and SEE share the highest amino acid sequence homology of 53 - 81%. SEB and the SECs are 50 - 66% homologous, while among the SPE, SPEA is most like SEB and shares 51% amino acid homology [83]. The SECs are unique in that they contain a HEXXH motif that represents the active site of thermolysin-like, Zn^{+2} metalloproteases such as those produced by *Bacillus anthracis* (i.e., lethal factor of anthrax toxin) and *Clostridium botulinum* (i.e., botulinum neurotoxins) [15]; however, there is evidently no proteolytic activity associated with the SECs. Interestingly, this motif (and slight variations of) follows a highly conserved region within SEA, SEB, SEC, SED, SEE and SPEA. Perhaps the HEXXH sequence in SECs, which was likely found in other SE, represents a primordial vestige of a previous activity commonly associated with these bacterial superantigens that now serves no useful function for *S. aureus*.

Although sequence similarities exist between these toxins, there are noticeable variations in stability towards heat and proteolysis. In contrast with the SE and SPE is TSST-1, which possesses a distinct, shorter sequence of 194 amino acids without the disulfide loop found in SE and SPE. TSST-1 is very resistant towards heat (1 h/100°C) or prolonged treatment with trypsin, whereas the SE are relatively intermediate in resisting heat and proteolysis [15]. In contrast, the biological activity of SPEA is readily destroyed by elevated temperatures (15 min/100°C) [84].

Despite differences in their amino acid sequences, structural studies reveal two highly conserved globular domains comprised of an N-terminal β-barrel and C-terminal β-grasp motif. [85,86]. X-ray crystallographic analyses of SEA, SEB, SEC2, TSST-1, SPEA, and SPEC more definitively reveal a conserved structure with two tightly packed domains containing both β-sheets and α-helices [87-95]. A shallow groove separating the two domains is considered the TCR-binding site and is relatively conserved among these bacterial toxins [92,96,97]. As described later, structure-function studies with site-directed mutagenesis and overlapping peptides of these superantigens, along with crystallographic analysis of superantigen/HLA-DR complexes, provide further

information on specific residues and peptide regions important for binding to MHC class II and TCR.

Additional indicators that SE, TSST-1, and SPE do share similar structures are evidenced by cross-reactivity and neutralization [54,98]. Historically, the SE were considered serologically distinct as determined by antisera and relatively insensitive immunodiffusion assays. However, subsequent studies employing the more sensitive ELISA with polyclonal and monoclonal antibodies suggest that common epitopes exist among these toxins [98-101], including cross-reactivity between SE and SPE [102,103].

Binding to MHC class II

Staphylococcal and streptococcal superantigens bind to conserved elements of MHC class II molecules with high affinity ($K_d = 10^{-8}$ - 10^{-6} M) (Table 2) [2,104-110].

Table 2. Properties of staphylococcal and streptococcal superantigens

Superantigen	K_d (nM)	MHC class II preference	Vβ specificity for human TCR
SEA	36/1000	HLA-DR > DP, DQ	1.1, 5.3, 6.3, 6.4 6.9, 7.3, 7.4, 7.9, 9.1, 18
SEB	244	HLA-DR > DQ > DP	3, 12, 13.2, 14, 15, 17, 20
SEC1	740	HLA-DR, DQ	3, 6.4, 6.9, 12, 15
SEC2	1000	HLA-DQ > DR	12, 13.1, 14, 15, 17, 20
SED	ND	HLA-DR > DP	5, 12
SEE	ND	HLA-DR > DP	5.1, 6.1- 6.4, 6.9, 8.1, 18
TSST-1	440	HLA-DR > DQ > DP	2
SPEA	104	HLA-DQ > DR > DP	2, 8, 12, 14, 15
SPEC	70	HLA-DR > DQ	1, 2, 5.1, 10
SPEG	16/1000	ND	2.1, 4.1, 6.9, 12.3
SPEH	37/2000	ND	2.1, 7.3, 9.1
SPEI	ND	ND	5.3, 6.9, 9.1, 18.1
SPEJ	ND	ND	2, 3, 8, 12, 14, 17

K_d values and MHC class II preferences are obtained from Proft et al. [81] and Mollick et al [105]. Two values listed for one toxin represent the high and low affinity sites, respectively.

Human Vβ specificities are taken from Kotzin et al. [1], Proft et al. [81], and Irwin et al. [60].

ND = not determined

However, each toxin displays preferential binding to distinct alleles of specific MHC isotypes, suggesting different sites and/or modes of contact for superantigen with MHC class II [105, 111-116]. Generally, HLA-DR binds SE and TSST-1 better than HLA-DP or -DQ, while murine IE molecules bind better than IA. In contrast to SE and TSST-1, the preferential binding of SPEA to HLA-transfected L cells is HLA-DQ > -DR > -DP [117,104]. Competitive binding studies show that SEA, SED, and SEE compete with SEB and TSST-1 for HLA-DR but SEB and TSST-1 do not inhibit binding of SEA, SED, or SEE [114,115,118]. SEB and TSST-1 have overlapping binding sites but do not compete with each other for HLA-DR or –DQ [119]. Thus, there are at least two different binding sites on MHC class II molecules for SE and TSST-1. A common overlapping binding region exists on HLA-DR for these toxins, referred to as the generic MHC class II binding site involving the α chain, but there is also an additional binding region for the SEA subfamily (SEA/SED/SEE/SEH).

Of the staphylococcal superantigens, SEA has the highest affinity for HLA-DR and bears two separate binding sites [114,120-124]. The higher affinity site is found within the C-terminus and binds to the HLA-DR β chain in a Zn^{2+} -dependent manner [120-122]. Another unique feature of this Zn^{2+} -binding site is that His81 of the DR1 β chain helps coordinate Zn^{2+} with three residues from SEA (His187, His226, and Asp227). The second site on SEA is of lower affinity, and like the binding site for SEB, it is located within the N-terminus (residue Phe47) and interacts with Gln18 found on the α chain of HLA-DR [123]. Other studies also indicate that one SEA molecule cannot interact with the α and β chains from the same MHC class II molecule [124,125]. Like SEA, a Zn^{2+} -binding motif is also present in SED as well as SEE, and coordination of Zn^{2+} in the SEA subfamily enables more efficient interactions with MHC class II molecules. Cross-linking of two MHC class II molecules by one SEA molecule is required for cytokine gene expression in a monocytic cell line [126], and dimerization of various staphylococcal superantigens like SEB, TSST-1 and/or class II molecules may play an important role in biological activity [127,128].

Like the N-terminus of the SEA family of toxins, the N-terminus of SEB, TSST-1, and SPEA has also been identified as a MHC class II binding site via biochemical and biophysical studies employing recombinantly altered toxins and monoclonal antibodies [96,97,129,130]. Co-crystal structures of SEB or TSST-1 complexed with HLA-DR1 further define the distinct differences in how these toxins bind to HLA-DR/peptide [127,128]. Although SEB and TSST-1 share the same interaction residues on the HLA-DR1α chain, they differ in their binding mode. SEB interacts exclusively with the α chain of HLA-DR1 and is unaffected by the HLA-associated peptide, unlike TSST-1 that also interacts with the α and β chains of HLA-DR1 or murine IA, as well as the C-terminus of certain bound peptides [131-134]. The peptide bound to MHC class II may not actually facilitate binding of TSST-1; however, it may effectively block TSST-1 interactions with MHC class II [134]. SEB and SPEA competitively bind to HLA-DR and studies have shown that analogous N-terminal regions of SPEA (residues 42 - 48) and SEB (residues 45 - 51) are important for class II interactions [104,127,135]. SPEC can interact with MHC class II solely through a high affinity Zn^{+2}-dependent site [94]. SPEC dimerizes in solution and can act as a bivalent ligand, binding to the β chains of two MHC molecule via Zn^{+2} at either end of the dimer [89]. This novel mode of cross-linking MHC class II is also seen in SPEJ [109]. The Zn^{+2}-dependent MHC class II

interactions of SPEH appear to be a hybrid whose N-terminal domain is most closely related to the SEB sub-family and the C-terminus resembles that of SPEC [95]. SPEI is most like SPEH and SEI in binding exclusively to the polymorphic MHC class II β-, but not α-, chain in a Zn^{+2}-dependent manner [109].

TCR interactions

Various X-ray crystallography studies clearly show two distinct and conserved domains within the SE, TSST-1, and SPE [87-93]. The groove formed between these domains represents the interaction site for the TCR Vβ chain [96,97,127,128]. These toxins each bind to a distinct repertoire of Vβ-bearing T cells, thus revealing a biological fingerprint amongst this toxin family [84,109,136,137]. Mutational analysis of SEB identifies the conserved residue Asn23 of SEB (Asn25 of SEA) and residues 60 - 64 as essential for recognition to murine TCR Vβ [97]. Mutations in the MHC class II binding domains of SEA differentially affect their interaction with TCR Vβ [138]. Others have found that a small increase in superantigen affinity for MHC class II can overcome a large decrease in toxin affinity for specific TCR Vβ [139].

Superantigen associated with MHC class II can bind directly to the soluble Vβ chain of TCR in the absence of Vα [140]. Contact between most superantigens and TCR occurs via the main-, not side-, chain residues of TCR Vβ [80,141,142]. As each toxin uniquely interacts with specific regions of the TCR Vβ chain, as demonstrated by different Vβ specificities of the highly homologous SEA subfamily, it is likely that MHC class II/TCR contacts differ for each toxin. Thus, for certain TCR Vβ, both the orientation of superantigen with the α chain of MHC class II and binding affinity for MHC molecules affect toxin/TCR interactions [123,127,128,143]. The binding affinity between TCR and SEB is relatively weak but strengthened by prior binding of SEB to MHC class II [144]. A cooperative effect is also observed between SEA, TCR, as well as MHC class II, thus enhancing complex stability [145]. This strengthens superantigen interactions such that they mimic TCR binding to a conventional MHC-peptide complex [143,145]. Therefore, the mitogenic potential of these toxins optimally results from a cooperative process involving binding of the superantigen/MHC complex to TCR with a resultant higher affinity than toxin alone.

In the case of TSST-1, T-cell activation may be influenced by APC-presented peptides because of the contacts made by the C-terminus of TSST-1 with peptide lying in the antigen-binding groove of HLA-DR [128]. Specifically, histidines 132, 135, and 140 of TSST-1 are important for TCR interactions and proinflammatory cytokine production, as demonstrated by in vitro and in vivo studies showing that these recombinantly altered molecules may also represent potential vaccine candidates [44,48,53,146-149].

Co-stimulatory molecules

The physiologic stimulation of T cells by conventional antigens involves at least two activation signals from APC [150]. The first signal comes from specific presentation of an immunogenic peptide bound to MHC class II on an APC to TCR. A second signal, resulting from the interaction of other receptors on APC and T cells, is needed for complete T-cell activation. The best characterized co-stimulatory receptors are

CD80/CD86 on APC and CD28/CTLA-4 from T cells [150,151]. Other co-stimulatory molecules such as CD40 on APC and CD154 on T cells are also important in modulating immune responses to conventional antigens [152,153]. Recognition of the superantigen/MHC complex by the TCR, and interaction of several co-stimulatory receptors present on both APC and T cells are also required for optimal activation of T cells by superantigens [151]. Expression of ICAM-1 on an APC plays an important role in promoting a stable cell conjugate and providing co-stimulatory signals. The interactions of LFA-1/ICAM-1 and CD28/CD80 have both been implicated in SEA-mediated T-cell activation [122]. This agrees with an observation that activation of the CD28-regulated signal transduction pathway during superantigen stimulation of T cells contributes to the induction of IL-2 expression [154]. A recent report suggests upregulation of CD80 and CD86 on monocytes and CD154 on T cells following exposure to TSST-1, but not with a recombinantly altered TSST-1 that is unable to induce cytokines [155]. Other surface molecules such as CD2, CD11a/ICAM-1, and ELAM are also implicated in the activation of human endothelial cells and T cells by SEB [156].

Signal transduction and in vitro cellular responses

The engagement of superantigen/MHC class II by TCR molecules results in signaling via MHC [157]. Like other mitogens, high concentrations of TSST-1 or SEB incubated with non-proliferating T cells increases phosphatidyl inositol levels and intracellular Ca^{2+} movement that activates the protein kinase C (PKC) pathway which is important for IL-2 expression [158,159]. In addition to the PKC pathway, superantigens also activate the protein tyrosine kinase (PTK) pathways that elevate expression of proinflammatory cytokines [157,160-163]. Activation of PKC and PTK is also required for enhanced cell adhesion observed after lymphocyte activation with MHC class II ligands. Staphylococcal superantigens also potently activate transcriptional factors NF-κβ and AP-1, which subsequently induce production of proinflammatory cytokines [164,165].

The concentration of SE and TSST-1 required for T-cell proliferation does not always correlate with their affinities for HLA-DR [105,114]. For instance, SEE binds HLA-DR with a 100-fold lower affinity relative to SEA but SEE stimulates T-cell proliferation at the same concentration as SEA [114]. In vitro, the dose response curves for cytokine and chemokine production by staphylococcal superantigen-stimulated cells were also very similar despite a difference in affinity/specificity of the various superantigens for MHC class II and TCR Vβ molecules [166]. These observations indicate that biological effects of staphylococcal superantigens are induced at rather low, nonsaturating occupancy rates and perhaps "low affinity" binding to MHC class II is most relevant to T-cell activation. Similar results have also been reported for SPEG [81], but with SPEA there does appear to be a direct correlation between binding affinity to HLA-DQ and mitogenicity [104].

Human peripheral blood mononuclear cells (PBMC) are commonly used to study the cellular requirements for activation by staphylococcal superantigens, and therapeutic agents have been designed to block these pathways [166-174]. PBMC secrete IL-1, -2, -6, TNFα, IFNγ, macrophage inflammatory protein 1α (MIP-1α), MIP-1β, and monocyte chemoattractant protein-1 (MCP-1) in response to SE, TSST-1, and SPE. Although

monocytes produce many chemokines and proinflammatory cytokines like IL-1, IL-6, and TNFα, added T cells enhance the mediator levels and suggest that cognate interaction of superantigen bound on APC with T cells contributes to cytokine and chemokine production [166,175]. There are contradictory reports of whether APC or T cells alone respond to these toxins without the other cell type, but production of these mediators by human monocytic cell lines or freshly isolated monocytes devoid of T cells has been reported [176,177]. However, others have found that IL-1 and TNFα induction by monocytes responding to SEA is strictly T-cell dependent [178]. SEA activation of human T cells is absolutely dependent on accessory cells [175]. Purified human T cells increase their expression of mRNA for several cytokines after superantigen exposure without APC, but the secretion of these cytokines and T-cell proliferation is dependent upon cells bearing MHC class II [179]. This is in agreement with TSST-1 studies showing that superantigen-induced production of cytokines is dependent upon APC [180]. However, high concentrations of SEC or SEE can induce T-cell proliferation and generate cytotoxic T cells in class II-deficient mice [181] while SEB can directly stimulate $CD8^+$ cells to lyse SEB-coated target cells [182]. It is possible that MHC class II is required for SE stimulation of most T cells but those possessing certain TCR Vβ can respond independently, but perhaps not as efficiently [183]. An analysis of SPEA-stimulated PBMC reveals that certain donors concomitantly produce low levels of monokines and T-cell cytokines [184]. Therefore, it appears that similar to the SEs, the ternary complex formed by SPE binding to MHC class II and TCR optimizes the activational signals for both APC and T cells. Direct presentation of superantigen to T cells without MHC class II molecules can evidently induce anergy [185]. It is likely that the cognate interaction of TCR with superantigen/MHC and that of other co-stimulatory receptors is required for maximum activation of T cells and APC, as well as regulation of cytokine and chemokine production by these cells.

Other cell types that respond to superantigens include B cells and synovial fibroblasts. Cross-linking of TCR with MHC class II on B cells by superantigen reportedly triggers B cell proliferation and differentiation into Ig-producing cells [186]. However, this effect is quite dose dependent in that a high concentration of superantigen can also inhibit Ig production. The CD28 co-stimulatory pathway plays a prominent role in superantigen-induced differentiation of B cells. TSST-1 reportedly suppresses Ig secretion from B cells possibly via apoptosis [43], thus hampering development of protective immunity against this toxin [7]. This effect may also be intimately linked to recurring susceptibility of TSS among patients [37,39-41]. Stimulation of synovial fibroblasts by superantigens also induces chemokine gene expression, raising the possibility that superantigens can trigger chemotactic responses and initiate or augment an inflammatory process like arthritis [187,188].

In vivo effects

In humans and non-human primates, SE induce an emetic response and toxic shock when ingested in microgram quantities [16,189]. In contrast, TSST-1 does not elicit emesis upon ingestion but it can cause systemic toxic shock via *S. aureus* growth on mucosal surfaces [35]. Unlike a number of other enterotoxins, specific cells and receptors in the intestinal tract have not been clearly linked to SE intoxication. Stimulation of mast cells and the release of cysteinyl leukotrienes, which can be blocked

by a receptor antagonist [190], appear to be responsible for emesis and skin reactions in primates [18,191]. Murine $V\beta8^+$ T cells found within Peyer's patches are adversely affected by orally administered SEB, as determined by a lack of in vitro stimulation towards SEB, thus suggesting an immune link with orally ingested SEB [192,193]. This may also explain earlier results by Sugiyama et al. [194] showing that non-human primates orally given a specific SE are transiently resistant to a subsequent higher dose of the same toxin. However, this oral resistance is not evident when these animals are given another SE serotype, a result likely linked to toxin-specific stimulation of unique Vβ-bearing T cells. In addition to toxin-specific resistance elicited by a single oral dose of SE, chronic intravenous exposure to SEA can virtually delete all Vβ-reactive T cells in mice [195]. Another study shows that mice intranasally administered a 1 μg dose of SEA (given once a week for 3 weeks), but not a recombinant SEA lacking superantigenicity, become resistant to a subsequent lethal challenge with SEA but not TSST-1 [196]. This resistance is evidently not due to toxin-specific antibody or depletion/anergy of SEA-reactive T cells; however, there is a significant increase in serum IL-10 levels among these animals and this correlates with previous in vitro and in vivo studies showing that IL-10 affords protection against SE-induced effects [167,197,198]. It has also been reported that footpad injections of SEB in mice can elicit a dose-related tolerance among $V\beta8^+$ T cells [199]. However, a potent enterotoxin (cholera toxin) produced by *Vibrio cholerae* and commonly used as an experimental mucosal adjuvant because it breaks antigen tolerance, can prevent SEB-induced non-responsiveness among T cells in Peyer's patches [200]. The specific mechanism(s) of how cholera toxin alters the SEB effects upon the mucosa remains a mystery.

Various investigators have attempted to locate a specific emetic domain within the SE; however, this still remains somewhat enigmatic. Studies with human Caco-2 colon monolayers reveal transcytosis of SEA, SEB, and TSST-1, and in vivo results from mice show that SEB enters the bloodstream more readily than SEA following ingestion [201]. These data suggest that the SE can cross the gastric mucosal barrier and circulate throughout the body. In vitro, these toxins do not act as cytotoxins that directly disrupt human intestinal cells (Henle 407), as evidenced by cell leakage or inhibition of protein or nucleic acid synthesis [202]. However, when a monolayer of another human line (T84 colonic cells) is incubated with SEB and PBMC, there is an increased ion flow suggesting that these toxins may indirectly affect the gut mucosa via the immune system [203]. A cytokine known to protect against various bacterial superantigens in vivo [198,204], IL-10, dose-dependently inhibits increased permeability when added before or concomitantly with the SEB co-culture [203].

It appears that MHC binding may not play a role in SE enteric effects, as recombinant variants of SEA (Leu48Gly) and SEB (Phe44Ser) devoid of MHC binding activity and T-cell mitogenicity are still emetic [205]. The disulfide loop of various SE, which is absent in TSST-1, may be responsible for the emetic activity of SE but that still remains equivocal [206,207]. Carboxymethylation of histidines on SEA [208] or SEB [209] generates toxin molecules devoid of enterotoxicity, or skin reactivity [191,210], yet they still retain superantigenicity. This chemically modified SEB can also inhibit the emetic/diarrheic effects of wild-type SEB in non-human primates when given concomitantly [210]. The lack of enterotoxicity attributed to carboxymethylated SEA is not due to an altered conformation and increased susceptibility to degradation by gastric

proteases [211]. Further analysis of each histidine in SEA-induced emesis and superantigenicity reveals that His61 is important for emesis but not T-cell proliferation, thus demonstrating that emesis and superantigenicity represent separate properties [211]. Another group used antibodies against a peptide region of SEA encompassing residues 121-180, which lacks the disulfide loop (Cys91-Cys105) and histidines, but these antibodies nonetheless prevent SEA-induced emesis [212].

The dual affinity of superantigens for MHC class II molecules and selected TCR Vβ enables these microbial toxins to perturb the immune system and induce high levels of proinflammatory and Th1-type cytokines [1,167-170,213,214]. Thus, SE, TSST-1, and SPE are pyrogenic in humans, non-human primates, and rabbits [26,32,215,216], a likely result of elevated levels of proinflammatory cytokines such as the synergistic acting IL-1 and TNFα from PBMC [217]. Both of these cytokines are endogenous pyrogens and induce fever by acting on the hypothalamus [218]. In addition, the circulating levels of other T-cell cytokines such as IFNγ, IL-2, and IL-6 are also increased after toxin exposure. IFNγ augments immunological responses by increasing the MHC class II levels on APC, as well as epithelial and endothelial cells. Additionally, IFNγ also upregulates TNFα and IL-1 receptors, thus acting synergistically with TNFα and IL-1 to increase expression of adhesion molecules on endothelial cells and subsequently promote leukocyte adhesion and recruitment. Superantigenic shock results from a crescendo of biological effects elicited by proinflammatory cytokines that adversely affect various organs such as the lungs [219].

Mice are often used as a model to study the immunological mechanisms of superantigen-mediated shock [220-226]. Although these animals lack an emetic response, they are ideal to work with regarding costs for in vivo screening of potential vaccines and therapeutics. However, these animals are naturally less susceptible to SE, TSST-1, and SPE (versus humans) because of the lower toxin affinity to murine MHC class II molecules [4,226]. Potentiating agents such as D-galactosamine, actinomycin D, lipopolysaccharide (LPS), or viruses have often been used to amplify the toxic effects of superantigens so that practical, lower amounts of toxin can be used for in vivo studies [221-224,227-230]. Many of our SE and TSST-1 studies have been accomplished with an LPS-potentiated mouse model, as it has been well established in various in vitro and in vivo systems that a natural synergy exists between these bacterial exotoxins and LPS [221,231-236]. As little as 2 μg of LPS in humans can cause endotoxic shock [237], and because bacterial superantigens like SE, TSST-1, and SPEA can synergistically augment the effects of LPS many log-fold, only picogram quantities of LPS in conjunction with a pyrogenic superantigen are needed to cause significantly severe effects [215]. When one considers the numbers of gram-negative bacteria in normal intestinal flora, and an increase in these microorganisms among TSS patients, the odds of this synergy naturally occurring are really quite high [215,238,239]. All of these studies show a correlation between increased serum levels of IL-1, IL-2, TNFα, and/or IFNγ with SEA-, SEB-, or TSST-1-induced shock [1,44,58,213,222,227]. Additionally, these efforts correlate nicely with others employing SEA and genetic knockout mice deficient in TNF p55 receptor or IFNγ [198]. Transgenic mice expressing human HLA-DQ6 and CD4 succumb to normally sublethal amounts of SEB (with D-galactosamine potentiation) and the serum TNFα levels among these transgenics correlate with lethal shock [240]. A more recent study also nicely reveals that transgenic mice expressing human HLA-DR3

and CD4 lethally respond to SE without a potentiating agent, thus providing a "simpler" model for future in vivo toxin studies [241]. PBMC isolated from naïve HLA-DR3-CD4 positive animals, when incubated with SEB in vitro, produce a marked increase of IL-6 and IFNγ relative to cells isolated from BALB/c mice, thus suggesting that proinflammatory cytokines also play a role in this murine shock model. Similar studies have also been done by this same group with SPEA and mice expressing human HLA-DQ8-CD4 [242]. Animals that overexpress murine TCR Vβ3 also have increased mortality linked to elevated TNF and IFNγ expression following infection by SEA-producing *S. aureus* [243]. Clearly, genetic manipulations of mice that yield expression of human HLA and CD4, or increased levels of specific murine TCR, can benefit future in vivo endeavors on superantigen-mediated pathogenesis.

A variation on lethality as an endpoint for superantigen intoxication is temperature. Historically, rabbits have afforded a nice in vivo model for SE-, SPE- or TSST-1-induced shock by using temperature or lethal endpoints [227,235,244-247]. Goats have also been used for studying the in vivo effects of TSST-1 and SEB, which include fever, after intravenous administration [248]. As evidenced in humans with TSS [231], rabbits given TSST-1 or SEB also have elevated levels of circulating LPS that can be eliminated along with the clinical signs of TSS by using polymyxin B [231,249,250]. The increased levels of circulating LPS may be due to impaired liver clearance by these protein exotoxins [235,251]. More recently, temperature has been used in LPS-potentiated mice implanted with a subcutaneous transponder [198] or telemetry device [252] for studying SE and TSST-1-induced shock. Results from these studies show a rapid temperature decrease readily evident among intoxicated mice within 10 h, thus giving an investigator a quick, non-lethal parameter for investigating exo- or endo-toxin induced effects. None of these studies reveal a temperature increase, thus suggesting a very rapid onset of shock in this LPS-potentiated murine model. In addition to the use of non-human primates, rabbits, goats, or mice for studying bacterial superantigens in vivo, a ferret model for oral SEB intoxication has also been reported which elicits emesis and rapid fever [253]. However, this latter model employs milligram, not microgram, quantities of toxin that are used in either murine or non-human primate models, perhaps making its use for vaccine or therapeutic discovery less feasible.

The role of individual cytokines or cellular receptors in mediating superantigen-induced shock was clarified recently by using genetic knockout mice lacking a specific cytokine, cytokine receptor, or co-stimulatory/adhesion receptor. Table 3 summarizes the results of genetic knockout mice deficient in IL-10, TNF receptor type I (TNF-RI) or type II (TNF-RII), CD28, CD43, CD95 or perforin [198,204,254-260]. The role of endogenous IL-10 in superantigen-mediated toxic shock was clarified by the use of IL-10 deficient mice, which are more susceptible to SEB-induced lethal shock versus wild-type controls [198,204]. Higher levels of proinflammatory mediators (i.e., TNFα, IFNγ, IL-1, -2, -6, -12, MIP-1α, and MIP-2) are observed in the serum of IL-10 deficient mice versus wild-type controls. The critical role of TNFα in staphylococcal superantigen-mediated shock was also confirmed using TNF-RI deficient mice [198,255]. Like many ligands, TNFα cannot exert a biological effect without its receptor and the TNF-RI deficient mice are protected against SEA- or SEB-induced shock. Similarly, mice lacking the co-stimulatory receptor CD28 are resistant to SEB-induced shock [256,257], perhaps due to the absence of serum TNFα after toxin exposure.

Table 3. Effects of specific genes on susceptibility of knock out mice to staphylococcal superantigens

Targeted gene	Effect on susceptibility to superantigen-induced shock
IL-10	Increased susceptiblity to SEA or SEB-induced shock, higher serum levels of TNFα, IL-1, IL-2, IL-6, IL-12, MIP-1α, MIP-2, IFNγ [198,204]
TNF-RI	Protection against SEA or SEB-induced shock [198,255]
TNF-RII	Slightly decreased susceptibility to SEA-mediated shock [198]
CD28	Protection against TSST-1-induced toxic shock syndrome [256] Protection against lethal toxic shock induced by second injection of SEB, lower serum TNFα [257]
CD43	Increased T-cell proliferation in vitro, enhanced homotypic adhesion [258]
CD95	In MRL-lpr/lpr mice, increased susceptibility to SEB-induced shock [259]
perforin	Decreased lysis of MHC class II-positive APC by SEA-activated $CD8^+$ T cells [260]

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Injection of SEB in mice has also been used to study activation-induced apotosis and T-cell anergy in vivo. This may be linked to a rapid (within 1 h) loss of L-selectin on the surface of specific Vβ-bearing T cells, thus resulting in decreased signal transduction [261,262]. Others have discovered that via endocytosis, surface levels of TCR-CD3 decrease ~50% among Vβ-reactive T cells within 30 min after SEB exposure [263]. The rapid hyperactivation and proliferation of T cells in mice following an SEB injection is transient, as within 48 h the majority of proliferating T cells are eliminated by activation-induced cell death [62,264]. These effects can render an animal incapable of mounting a primary immune response against another antigen 3 days after SEB exposure, even when given in Freund's adjuvant [226]. The adhesion receptor CD95 plays an important role in eliminating activated cells and the residual Vβ specific cells become anergic and functionally unresponsive. However, controversy still exists regarding the functional ability and fate of these anergic T cells. After injection of SEB into mice, splenic $V\beta8^+$ T cells are deleted or no longer respond to SEB and produce less IL-2 and IFNγ [265]. In contrast, others report that these anergic cells synthesize less IL-2 but secrete IFNγ that can mediate toxic shock following a subsequent dose of SEB [266]. An evident paradox is that the anti-inflammatory cytokine IL-10, which protects against SE-induced shock [167,197,198], is also produced by SEB-primed T cells and perhaps reflects an attempt by the host to counter the proinflammatory effects elicited by IFNγ. It is likely that SEB-induced anergy differentially affects $CD4^+$ and $CD8^+$ T cells,

with $CD4^+$ being more susceptible [266]. This may also explain why $CD8^+$ cytotoxic T cells are activated by superantigens and thus represent potential antitumor reagents [267].

Autoimmunity

The ability of superantigens to cross-link MHC class II molecules and specific TCR Vβ enables these microbial toxins to stimulate the immune system and induce autoimmunity by activating APC and normally quiescent, autoreactive T- and B-cells. Activation results in cytokine and chemokine release, thus mediating a potent inflammatory response. Several experimental animal models show that staphylococcal superantigens are arthrogenic, such as TSST-1 which exacerbates bacterial cell wall-induced arthritis in rats and is possibly linked to accumulation of $V\beta11^+$ T cells and IFNγ production within arthritic joints [268,269]. TSST-1 also plays a pivotal role in murine septic arthritis, as the frequency and severity of this disease are increased after intravenous administration of TSST-1-secreting *S. aureus* [270]. In humans, there is a good correlation between the presence of SEB-specific IgM and arthritis, thus suggesting a role for this toxin in disease [271]. SEA or SEB can also induce relapses of experimental autoimmune encephalomyelitis in a murine model for multiple sclerosis [272,273]. Experimentally, IFN-tau (a type I IFN) can cause remission of murine encephalomyelitis without apparent toxicity [274,275]. How exogenously administered toxin triggers autoimmune processes like arthritis is unknown, but it is likely that proinflammatory cytokines and chemokines produced in response to superantigens facilitate specific recruitment and migration of autoreactive T cells into synovial tissue and joints. However, IL-10 can play a protective role in maintaining a tolerant state [276]; therefore, self-induced proteins such as cytokines represent a dual edged sword in exacerbating or protecting the host against toxic superantigens. In the presence of minor tissue injury or inflammation and its attendant release of potential autoantigens, an increased presence of immune cells might initiate a destructive autoimmune reaction.

There is some evidence (albeit controversial) that TSST-1, or SPE, can elicit Kawasaki syndrome which evidently involves an autoimmune mechanism(s) [277-280]. However, a recent study found no statistical significance in the overall isolation rate of bacteria producing superantigens from Kawasaki disease and other febrile patients [280], thus there is no consistent, overwhelming data to date suggesting linkage of *S. aureus* or *S. pyogenes* with this disease. Although no single causative agent has been unequivocally associated with Kawasaki disease, observed coronary artery lesions among patients are likely due to improper activation of the immune system. Patients with acute Kawasaki disease have higher serum levels of vascular endothelial growth factors [281], which can play a role in coronary artery abnormalities [282].

Psoriasis and atopic dermatitis also represent autoimmune diseases linked to staphylococcal and streptococcal colonization of skin and subsequent production of exotoxins like SEA, SEB, SEC, TSST-1, and SPE [283-286]. SEB on healthy human skin induces inflammatory reactions, which may be linked to degranulation of cutaneous mast cells as evidenced with non-human primates [191,287]. The T cells from patients with severe atopic dermatitis are apoptotic, which may lead to increasingly chronic infections and subsequent worsening of disease [288]. Serum IgE towards SEA, SEB, or TSST-1, which is rare among individuals without atopic dermatitis even if they are

colonized by toxin producing strains of *S. aureus* [289], may contribute to sequelae associated with disease and is linked to activation of $CD4^+$ T cells [285,289-293]. Bacterial density on the skin can affect whether an individual becomes sensitized towards these toxins and subsequently develops atopic dermatitis [294], thus hygiene, environment, resident normal flora, and antibiotic usage likely play important roles in survival and growth of these microbial offenders.

Therapeutics and vaccines

To date, there are no effective therapeutics or vaccines against SE, TSST-1, or SPE that have been approved for human use by the United States Food and Drug Administration. Regarding conventional immunotherapies towards these toxin types, there are three important targets: (1) TCR-toxin-MHC class II interactions; (2) any accessory, co-stimulatory, or adhesion molecule involved in activation and effector functions of T cells; and (3) cytokine release by activated T cells and APC. Inhibition of all or one of the above targets/pathways has been reported both in vitro and in vivo, thus representing viable means of curbing the biological effects of these toxins. For example, steroids and IL-10 were investigated as possible agents for inhibiting the production of proinflammatory cytokines and T-cell proliferation following TSST-1-stimulation of human PBMC in vitro [167]. Recently, Arad et al. [295] discovered that a conserved 12 amino acid region (150-161) of SEB prevents SEA, SEB, TSST-1, or SPEA-induced lethal shock in mice when given intravenously 30 min after the intraperitoneal toxin dose. Another group revealed that this peptide also prevents transcytosis of various SE and TSST-1 across a human colonic cell (T84) monolayer [296]. This segment of SEB is not associated with the classically defined MHC class II or TCR binding domains, but it may block co-stimulatory signals necessary for T-cell activation and clearly represents a potential target for vaccines and therapeutics against SE and SPE.

Several aforementioned in vivo models have been used to study the prevention of superantigen-induced shock. Therapeutic agents such as nitric oxide inhibitors can mitigate the effects of SEA and SEB by inhibiting the production of IL-1, -2, -6, TNF, and IFNγ [297,298]. Blockade of a co-stimulatory receptor, CD28, by its synthetic ligand, CTLA4-Ig, effectively prevents TSST-1-induced proliferation of T cells in vitro as well as lethal TSS in vivo [299]. This effect is likely mediated by $CD8^+$ T cells, as TSST-1 resistance could be transferred to naïve mice by using $CD8^+$ T cells from CTLA4-Ig-treated animals. Neutralizing antibodies against TNFα prevent SEB-induced lethality [213]. IL-10 can block the production of IL-1, TNFα, and IFNγ, resulting in reduced lethality from superantigen-induced toxic shock [197]. Recently, it was discovered that a novel nasal application of SEA in mice induces tolerance towards SEA, but not TSST-1 [196]. This phenomenon is evidently linked to increased serum levels of IL-10, but not depletion of SEA-reactive T cells or toxin-specific antibodies. Anti-inflammatory agents such as indomethacin and dexamethasone also effectively lower the febrile response in rabbits injected with SEA [246]. Predictably, anti-inflammatory agents simultaneously lower serum concentrations of IL-1, IL-6, TNFα, and IFNγ in SEA-treated rabbits. Studies with human PBMC in vitro and a LPS-potentiated mouse model show that drugs like pentoxifylline and pirfenidone lower expression of proinflammatory cytokines, thus abrogating the ill effects of SEB or TSST-1 [171,173].

Urinary trypsin inhibitor, a glycoprotein that blocks the activity of various serine type proteases, evidently binds to LPS and SEB which subsequently suppresses SEB-induced lung injury in rats [300]. SEA also causes an inflammatory reaction in the lungs, but a hexapeptide inhibitor of IL-8, a cytokine produced by human alveolar macrophages and stimulated by SEA, can decrease neutrophil influx into this organ [301-302]. Release of proinflammatory cytokines due to SEB or TSST-1 is also diminished in vivo by soluble β-glucans; however, the mechanism is unknown [303].

As previously mentioned, SE- and SPE-induced psoriasis is a T-cell linked autoimmune disease [285,286]. Recent efforts have shown that a SEA mutant devoid of MHC class II binding activity can act as a therapeutic towards SEA-induced psoriasis [286], perhaps by competing for T-cell binding. This effect is specific, as there is no competitive effect between the SEA mutant and wild-type SEB in a murine psoriasis model. It was also proposed that this non-toxic mutant of SEA represents a potential therapeutic vaccine to elicit antibodies that neutralize SEA-induced psoriasis, thus making it clinically important to diagnose what microorganism is involved in disease and the superantigens that it produces in situ.

A novel use of bacterial superantigens represents the activation of cytotoxic T cells and subsequent elevation of proinflammatory cytokine levels for tumor therapy. SEA alone [267], or a tumor-specific monoclonal antibody fused either to SEA [304,305] or recombinantly attenuated SEA deficient in MHC class II binding [306,307] represent promising reagents in animal models and humans. SEA, in conjunction with protein-A, also provides a potent anti-tumor response in mice that is better than either component alone [308]. The importance of stimulating $V\beta8^+/CD8^+$ T cells has been demonstrated in a mouse tumor model with melphalan and co-administered SEB, which further increases survival of tumor-bearing mice [309]. Studies have also shown that the C-terminus (residues 88-194) of TSST-1 stimulates anti-tumor effects in vitro and in vivo [310]. These, and other, exciting studies seemingly warrant further efforts to use bacterial superantigens as anti-tumor reagents.

In addition to the use of therapeutics to abrogate the ill effects of bacterial superantigens, work has been done by various groups to develop vaccines for staphylococcal and streptococcal superantigens. Preexisting antibodies towards the SE, TSST-1, and SPE can play an important role in disease outcome [39,40,311] and the use of intravenous Ig (IVIG) has proven useful in humans following the onset of staphylococcal or streptococcal toxic shock [312-314]. Given this wealth of information, it makes logical sense that vaccination might be useful for preventing TSS. Recombinantly attenuated mutants of SEA, SEB, TSST-1, SPEA, and SPEC, which do not bind MHC class II and/or specific Vβ TCR molecules, have been used successfully as vaccines to prevent toxic shock in different animal models [44-53,222]. These vaccines have been given either parenterally [44,45,47,49] or mucosally [46] and have proven efficacious against a toxin challenge or *S. aureus* infection. Other murine and non-human primate studies have used formalin-inactivated SEB and reveal protection towards a homologous toxin challenge after vaccine is given parenterally or mucosally [315-318]. However, others have demonstrated that formaldehyde treatment of proteins can adversely affect processing and subsequent presentation to the immune system [319], especially if the modified protein is given as a mucosal immunogen [320].

Summary

S. aureus and *S. pyogenes* produce various protein superantigens that represent important virulence factors which interact with MHC class II and TCR molecules on the surface of host cells. The host's response to SE, TSST-1, or SPE is what triggers the severe effects of these toxins, such as shock and possibly death, which are mediated via release of various proinflammatory cytokines. Similar amino acid homology and biological activities among this family of protein exotoxins suggest a common, and constantly evolving, endpoint via divergent and/or convergent evolutionary paths. With time, it is clear that more of these fascinating toxins will be discovered in other microorganisms and perhaps novel biological properties elucidated by future investigators. Since superantigens can afford an advantage to a microorganism, such as delayed clearance from a host [321], there is biological justification of the energy expended for transcription and translation of these genes by a procaryote. After an early cytokine "burst" and T-cell activation, the subsequent immunosuppression and T-cell anergy induced by SE, TSST-1, and SPE represent likely mechanisms that aid in survival of the invading pathogen. Interference with any of these component steps diminishes or prevents toxic manifestations of these superantigens and decreases the morbidity, and perhaps mortality, of an infected host. Various drugs and vaccines are being tested experimentally and represent novel means of protecting a susceptible population against toxin-producing, antibiotic-resistant *S. aureus* and *S. pyogenes*. A medical version of the "Maginot Line" (i.e. antibiotics) has been circumvented (vancomycin resistant *S. aureus*) and how we respond to these, and other, microbial threats will have lasting consequences for subsequent generations. From this immunologist's and microbiologist's perspective, the time is nigh to employ existing experimental data and use it as best we can to remain a step ahead of these constantly evolving pathogens and their intriguing superantigenic toxins.

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