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Chemotherapeutics targeting immune activation by staphylococcal superantigens

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Summary

Staphylococcal enterotoxin B (SEB) and related superantigenic toxins are potent activators of the immune system and cause a variety of diseases in humans, ranging from food poisoning to toxic shock. These toxins bind to both MHC class II molecules and specific Vβ regions of T cell receptors (TCR), resulting in the activation of both monocytes/macrophages and T lymphocytes. The interactions of these toxins with host cells lead to excessive production of proinflammatory cytokines and T cell proliferation, causing clinical symptoms that include fever, hypotension and shock. Different domains of SEB contributing to MHC class II or TCR interactions have been mapped and defined by mutagenesis, crystallography and other biochemical techniques. This review summarizes the *in vitro* and *in vivo* effects of staphylococcal superantigens, and the therapeutic agents to mitigate their toxic effects. Potential targets to prevent the toxic effects of bacterial superantigens include blocking the interaction of SEs with MHC or TCR, or other costimulatory molecules; inhibition of signal transduction pathways used by these superantigens; inhibition of cytokine and chemokine production; and inhibition of the downstream signaling pathways used by proinflammatory cytokines and chemokines. Early blockade of these targets proves to be useful *in vitro* and *in vivo* testing of therapeutics against SEB-induced toxic shock will also be reviewed.

key words: **staphylococcal superantigens • therapeutics • toxic shock**

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14. ABSTRACT
Staphylococcal enterotoxin B (SEB) and related superantigenic toxins are potent activators of the immune system and cause a variety of diseases in humans, ranging from food poisoning to toxic shock. These toxins bind to both MHC class II molecules and specific Vb regions of T cell receptors (TCR), resulting in the activation of both monocytes/macrophages and T lymphocytes. The interactions of these toxins with host cells lead to excessive production of proinflammatory cytokines and T cell proliferation, causing clinical symptoms that include fever, hypotension and shock. Different domains of SEB contributing to MHC class II or TCR interactions have been mapped and defined by mutagenesis, crystallography and other biochemical techniques. This review summarizes the in vitro and in vivo effects of staphylococcal superantigens, and the therapeutic agents to mitigate their toxic effects. Potential targets to prevent the toxic effects of bacterial superantigens include blocking the interaction of SEs with MHC or TCR, or other costimulatory molecules; inhibition of signal transduction pathways used by these superantigens; inhibition of cytokine and chemokine production; and inhibition of the downstream signaling pathways used by proinflammatory cytokines and chemokines. Early blockade of these targets proves to be useful in vitro and in vivo testing of therapeutics against SEB-induced toxic shock will also be reviewed.

15. SUBJECT TERMS
staphyloccus aureus, staphylococcal enterotoxin, SEB, superantigen, proinflmmatory cytokines, review

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BACKGROUND

Staphylococcus aureus produces several exotoxins, staphylococcal enterotoxins A through P (SEA-SEP), and toxic shock syndrome toxin 1 (TSST-1), which contribute to its ability to cause disease in humans and laboratory animals [1–4]. SEB is the most widely studied toxin among the staphylococcal exotoxins and is listed by the Centers for Disease Control and Prevention (CDC) as a category B priority agent as it can be used as an air-borne, food-borne and water-borne toxic agent. Depending on the dose and route of exposure, SEB causes food poisoning, acute and fatal respiratory distress and toxic shock [5–7].

The term "superantigen" is used to describe these microbial products because they activate a large proportion of T cells (5–30%) whereas a conventional antigen stimulates less than 0.01% of the T-cell population [3,8]. However, the interaction of superantigen with host cells differs from that of conventional antigens in that it binds outside the peptide-binding groove of MHC class II, exerts its effect as an intact molecule without being "processed", and its presentation to T cells is not MHC-restricted [3,4,9–13]. The dual affinity of staphylococcal superantigens for MHC class II molecules and specific T-cell receptor Vβ (TCR) chains enables these microbial toxins to perturb the immune system and induce high levels of proinflammatory cytokines and chemokines [1,7,14–19]. Two of these cytokines, tumor necrosis factor α (TNFα) and interleukin 1 (IL-1), are direct mediators of fever, hypotension, and shock [20].

SUPERANTIGEN STRUCTURE AND BINDING TO HOST CELLS

Staphylococcal enterotoxins (SEs) and TSST-1 are 22- to 30-kD single chain proteins and are grouped into three classes based on their primary sequence homology [1–3]. SEA, SED, SEE, and SEH share the highest sequence homology, between 53% and 81%. The second group consists of SEB, the SECs, and SEG, which are 50% to 66% homologous. TSST-1 is distantly related (28% homology) as it has a distinct, shorter primary sequence of 194 amino acids with no cysteines and a missing "disulfide loop" found in SEs. X-ray crystallographic analyses of SEB and TSST-1 show similarities in the general structure in that two tightly packed domains containing both β-sheets and α-helices are present in both SEB and TSST-1 molecules [21,22]. The TCR-binding site lies in the shallow groove between these two domains [21,23,24].

Superantigens bind to common, conserved elements of MHC class II molecules with each individual toxin displaying preferential binding to certain MHC isotypes indicating different modes of contact of superantigen with MHC class II binding sites [24–31]. There are two different binding sites on MHC class II molecules for staphylococcal toxins, a common low-affinity binding site on the MHC class II α chain and a high-affinity, zinc-dependent binding site on the β chain for the SEA subfamily [25]. The interaction of each toxin to the TCR Vβ chain is unique as shown by the different Vβ specificities of each superantigen [3,4]. The binding contacts are mostly between the side-chain atoms of the superantigen and the complementarity-determining regions 1 and 2 and the hypervarible region 4 of the Vβ chain. The mitogenic potency of these toxins is the result of a cooperative process such that the superantigen/MHC complex binds the TCR with a higher affinity than does toxin alone [26].

As with conventional antigens, the expression of costimulatory molecules on antigen-presenting cells (APC) and T cells provide additional signals for cell activation and can direct T cell differentiation into T helper type 1 (Th1) or type 2 (Th2) responses. The expression of intercellular adhesion molecule (ICAM) on an APC promotes stable cell conjugate formation and provides co-stimulatory signals. The interactions of LFA-1/ICAM-1 and B7/CD28 have both been implicated in SEA-mediated, T-cell activation [32]. The activation of the CD28-regulated signal transduction pathway during superantigen stimulation of T cells was reported to be necessary for the induction of IL-2 [33]. Other surface molecules such as CD2, CD11a/ICAM-1, and ELAM are also required for optimal activation of endothelial cells and T cells by SEB [34].

SIGNAL TRANSDUCTION PATHWAYS

The interaction of superantigen with MHC class II and TCR on APC and T cells leads to intracellular signaling [35]. High concentrations of SEB elicit phosphatidyl inositol production and intracellular Ca^{2+} flux in T-cell clones without inducing proliferation [36]. Other early activation events include activation of protein kinase C (PKC) and protein tyrosine kinase (PTK) pathways [35,37,38] similar to mitogenic activation of T cells. Superantigens also activate transcriptional factors NFκB and AP-1, resulting in the expression of proinflammatory cytokines, chemokines, and adhesion molecules [35]. The proinflammatory cytokines IL-1 and TNFα can directly activate the transcriptional factor NFκB in many other cell types including epithelial cells and endothelial cells perpetuating the inflammatory response [20]. Furthermore, IL-1 interacts with IL-1 receptor 1 (IL-1R1) activating downstream signaling molecules, the adaptor MyD88, IL-1R1 associated protein kinase (IRAK), and TNF receptor-associated factor 6 (TRAF-6) [20]. The similarity between the IL-1R signaling pathway and that of toll-like receptors (TLR) used by pathogens to stimulate innate immune system underscores the importance of controlling the cytokine cascade.

CELLULAR RESPONSES TO SUPERANTIGENS

Human peripheral blood mononuclear cells (PBMC) are used extensively to study the cellular requirements for activation by staphylococcal superantigen and therapeutic agents have been designed to block these pathways [14–19,39–41]. PBMC secrete the cytokines IL-1, IL-2, IL-6, TNFα, gamma interferon (IFNγ); the chemokines, macrophage inflammatory protein 1α (MIP-1α), MIP-1β, and monocyte chemoattractant protein-1 (MCP-1), in response to SEB and TSST-1 [15]. Monocytes produce most of the proinflammatory cytokines and chemokines [39]. However, adding T cells potentiates the levels of mediators induced, suggesting that cognate interaction of superantigen bound on APC with T cells contributes to the production of these cytokines and chemokines [39,42]. Direct superantigen presentation to T cells in the absence of MHC class II molecules can induce an anergic response [43].

Other cell types responding directly to staphylococcal superantigen include B cells, synovial fibroblasts, intestinal epithelial cells, and mast cells [44–47]. Stimulation of synovial fibrob-

lasts with superantigens leads to the induction of chemokine gene expression, raising the possiblity that superantigens can trigger chemotactic cytokines and initiate inflammatory arthritis [45]. SEB was shown to transcytosed across an intestinal epithelial cell line [46]. The interaction of superantigen with epithelial cells and endothelial cells is mostly indirect, i.e, through the release of IL-1, TNFα, and IFNγ from APC and T cells [48,49]. IL-1, TNFα, and IFNγ are key mediators released by immune cells in response to many inflammatory stimuli such as lipopolysaccharide (LPS), other bacterial cell wall components, and pathogens. Both IL-1 and TNFα are endogenous pyrogens and activate many cell types to enhance immune reactions and inflammation [20]. IFNγ produced by activated T cells augments immunological responses by increasing MHC class II and ICAM-1 on APC, as well as epithelial and endothelial cells [20]. Additionally, IFNγ upregulates TNFα and IL-1 receptors, and acts synergistically with TNFα and IL-1 to enhance immune reactions and promote tissue injury. The chemokines, IL-8, MCP-1, MIP-1α, MIP-1β induced directly by SEB and TSST-1 can selectively chemoattract and activate leukocytes [20,39].

Animal Models of Disease

In humans and monkeys, SEs induce an emetic response when ingested and intravenous injection of submicrogram concentrations causes fever, toxic shock and death [50]. Mast cell stimulation and the release of cysteinyl leukotrienes contribute to the emetic response [47]. TSST-1 causes systemic toxic shock but not emesis [1,2]. Thus the domain contributing to the emetic and superantigenic effects of these toxins appears to be separate and the disulfide loop of SEs, which is absent in TSST-1, is likely the domain responsible for the emetic activity of SEs. Aerosolized SEB in monkeys resulted in emesis and diarrhea developed within 24 hours of exposure, followed by the abrupt onset of lethargy, difficulty breathing and finally death from toxic shock.

Mice are less susceptible to the effects of SEs and TSST-1 as the affinity of these toxins to mouse MHC class II is much lower [51,52]. Potentiating agents such as D-galactosamine, actinomycin D, LPS, or viruses (infection) are often used to amplify the toxic effects of superantigens so that practical, lower amounts of toxin can be used to induce toxic shock [52–57]. LPS synergizes with superantigens in the induction of the proinflammatory cytokine cascade [52]. In these mouse models, a correlation exists between increased serum levels of IL-1, IL-2, TNFα, and IFNγ with SEB-induced shock [51,52,56,57]. It is likely that shock syndrome induced by superantigens results from the culminating biological effects of these proinflammatory cytokines.

Therapeutics for Superantigen-Induced Shock

Given the complex pathophysiology of toxic shock, an understanding of the interaction of cellular receptors and signaling pathways used by these staphylococcal superantigens and the biological mediators they induce would provide insights to selecting appropriate therapeutic targets. Thus, potential targets to prevent the toxic effects of SEs include blocking the interaction of SEs with MHC or TCR [35], or other costimulatory molecules [32–34,58]; inhibition of signal transduction pathways used by SEs [35,59]; inhibition of cytokine and chemokine production [39,41,60]; and inhibition of the downstream signaling pathways used by proinflammatory cytokines and chemokines.

An early study indicates that blockade of the CD28 co-stimulatory receptor by its synthetic ligand, CTLA4-Ig, effectively prevents TSST-1-induced proliferation of T cells *in vitro* as well as lethal toxic shock *in vivo* [58]. Other investigators reported the use of small peptides as antagonist to block SEB-induced cytokines and proliferation *in vitro* and *in vivo* [61,62]. Thus a conserved region of 12 amino acids (residues 150–161) from SEB prevents SEA-, SEB-, or TSST-1-induced lethal shock in mice when given intravenously 30 min after an intraperitoneal toxin dose [61]. 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. However a subsequent study of these peptides indicated that they are ineffective inhibitors of SEB-induced effects both *in vitro* and *in vivo* [63]. Recently, bi-specific chimeric inhibitors composed of the DRα1 domain of MHC class II and Vβ domain of the TCR connected by a flexible $GSTAPPA)_3$ linker were reported to bind SEB competitively and prevent its binding to MHC class II of APC and TCR on T cells [64].

Another important target is the induction of the proinflammatory cytokines and the signaling pathways used by superantigens. TNFα, IL-1, and IFNγ are key mediators in SEB-induced toxic shock, and *in vivo* studies also show a correlation between increased serum levels of these cytokines with SEB-induced lethality [52]. Genetic knockout mice deficient in TNF receptor type 1 (TNFR1) or IFNγ receptor were resistant to SEB-induced shock [65]. Neutralizing antibodies against TNFα prevented SEB-induced lethality [56]. The anti-inflammatory cytokine IL-10 was used to block the production of IL-1, TNFα and IFNγ, resulting in reduced lethality to superantigen-induced toxic shock [66]. The focus of our therapeutic efforts is to identify pharmacological agents, new or FDA-approved old ones, for preventing or treating SEB-induced shock. A list of agents, most of them low molecular weight compounds effective in blocking the effects of superantigens, is shown in Table 1 [60,67–76]. The steroid dexamethasone is a potent immunosuppressor and blocked SEB- and TSST-1-induced cytokines and T-cell proliferation [41]. Therapeutic agents such as nitric oxide inhibitors can mitigate the effects of SEB by inhibiting the production of IL-2 and IFNγ [68]. D609, a phospholipase C inhibitor, blocks SE-induced effects both *in vitro* and *in vivo* [71,72]. Another compound, baicalin, is a flavone isolated from the Chinese medicinal herb Scutellaria baicalensis, is a potent inhibitor of SEB-mediated effects *in vitro* [73]. Its use is currently under investigation *in vivo*. Another drug that we recently found to be anti-inflammatory and inhibited SEB-induced proinflammatory cytokines and chemokines is doxycycline [75]. Two of these compounds, pentoxifylline and doxycyline [60,75], are FDA-approved drugs used for other indications and have been in clinical use for many years. Recently cell-permeable peptides targeting NFκB were found to attenuate SEB-induced T cell responses in mice [77].

A major problem of *in vivo* testing of therapeutics against SEB-induced toxic shock is finding a relevant model that mimics human disease. Mice are generally preferred as experimental models for drug testing because of their inbred homogeneity and because large numbers of animals can be used with

Table 1. Therapeutics for inhibition of SEB-induced effects.

Drug/Compound	Biological Effects against SEB	Other Effects
Dexamethasone	Inhibits SEB-induced proinflammatory cytokines in PBMC [41] Inhibits cell adhesion molecules (ICAM, ELAM, VCAM) on endothelial cells [67]	FDA approved since 1940s, prescribed for treating inflammatory diseases
Niacinamide	Inhibits SEB-induced lethality in mice [68]	Inhibits nitric oxide synthetase
Pentoxifylline	Inhibits SEB-induced proinflammatory cytokines and chemokines at transcriptional level [39,60] Blocks SEB-induced proliferation Inhibits ICAM expression on pulmonary epithelial cells [69]	FDA approved since 1970s, prescribed for treating peripheral arterial disease
TJU103-stucture designed by computer	Inhibits SEB-induced cytokines and T cell proliferation in human PBMC [70]	Interferes with CD4
D609	Blocks SEB-stimulated cytokines, chemokines and proliferation in human PBMC and SEB-challenged mice [71,72]	Phopholipase C inhibitor
Baicalin	Inhibits SEB-induced cytokines, chemokines at the transcriptional level [73] Blocks SEB-induced proliferation Inhibits NFκB activation [73]	Chinese herbal medicine, used in China and Japan to treat infectious diseases
Pirfenidone	Inhibits SEB-stimulated cytokines *in vitro* and *in vivo* Blocks SEB-stimulated T cell proliferation [74]	Anti-fibrotic agent, inhibits TGFβ
Doxycycline	Inhibits SEB-induced cytokines and chemokines in human PBMC Blocks SEB-induced T cell proliferation [75]	FDA approved antibiotic used to treat bacterial infections
Caspase Inhibitor	Inhibits SEB-induced cytokines, chemokines and proliferation in human PBMC [76]	Prevents apoptosis

results available in a relatively short time. However, mice are poor responders to SEB and are naturally resistant to superantigen-induced toxic shock. The mouse models used relied on the use of sensitizing agents such as D-galactosamine, actinomycin D, or LPS. Recently transgenic mice with human MHC class II were found to be an ideal animal model for examining the biological effects of superantigens as they respond to much lower doses of toxins due to the higher affinity binding of SEs to human MHC class II [78]. More studies are underway with transgenic mice in the therapeutic discovery of inhibitors to counteract the effects of superantigens.

Antibody-based therapy targeting direct neutralization of SEB or other superantigen represents another form of therapeutics most suitable at the early stages of exposure before the activation of cells and the release of proinflammatory cytokines [79]. Some of these neutralizing antibodies against superantigens cross-reacted among different superantigens. Mutants with altered critical residues in SEB involved in MHC class II binding of SEB were also used successfully to vaccinate mice and monkeys against SEB-induced disease [80].

The views expressed in this publication are those of the authors and do not reflect the official policy or position of the Department of the Army, the Department of Defense, or the U.S. Government.

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