



Published in final edited form as:

Microbiol Spectr. 2013 December ; 1(2): . doi:10.1128/microbiolspec.AID-0002-2012.

Bacterial Toxins—Staphylococcal Enterotoxin B

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Abstract

Staphylococcal enterotoxin B is one of the most potent bacterial superantigens that exerts profound toxic effects upon the immune system, leading to stimulation of cytokine release and inflammation. It is associated with food poisoning, nonmenstrual toxic shock, atopic dermatitis, asthma, and nasal polyps in humans. Currently, there is no treatment or vaccine available. Passive immunotherapy using monoclonal antibodies made in several different species has shown significant inhibition in in vitro studies and reduction in staphylococcal enterotoxin B-induced lethal shock in in vivo studies. This should encourage future endeavors to develop these antibodies as therapeutic reagents.

INTRODUCTION

Staphylococcal Enterotoxins

Staphylococcus aureus is a nonmotile, ubiquitous, gram-positive coccus which is a major human pathogen responsible for a wide range of infections, including skin and soft tissue infections, bacteremia, pneumonia, and several toxin-mediated diseases. Among many extracellular proteins, *S. aureus* strains also secrete a variety of potent toxins which include alpha hemolysin, enterotoxins, leukocidins, and exfoliative toxins, all of which are directly associated with particular disease manifestations. To date, more than 33 enterotoxin sequences have been described in various *S. aureus* genomes. Enterotoxins are heat stable and exert their effect on the epithelium of the intestinal tract when ingested, and thus, they are a common cause of food poisoning. Several enterotoxins are potent superantigens (SAGs) that, in a non-antigen (Ag)-dependent way, predominantly activate CD4⁺ T cells (1) but also activate other immune cells. The SAGs of *S. aureus* include toxic shock syndrome toxin 1 (TSST-1), enterotoxin serotypes A to E and I (*sea*, *seb*, *sec*, *sed*, *see*, and *sei*), and enterotoxin-like serotypes G (*selG*), H (*selH*), and J to U (*selJ* to *selU*). Of these SAGs, *sea* to *see* have the ability to induce emesis in monkeys and are thus referred to as classic enterotoxins. The remaining SAGs either have not been tested for emetic activity or lack emetic activity and are therefore referred to as enterotoxin-like proteins (*selG*, *selH*, and *selJ* to *selU*). For the most part, staphylococcal SAGs are encoded by mobile genetic elements, which include extrachromosomal plasmids as well as chromosomal prophages, transposons, and pathogenicity islands. It is noteworthy that a chromosomally carried enterotoxin-like

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Conflicts of interest: We disclose no conflicts.

gene (*seIX*) was recently identified (2). The *seb* gene is carried on the pathogenicity island SaPI3. The genes of SAgS *selG*, *selI*, *selM*, *selO*, and *selU* are located in the enterotoxin gene cluster (*egc*) and are among the most prevalent SAgS in clinical *S. aureus* isolates. They are expressed by *S. aureus* during logarithmic growth and shut off expression once a certain bacterial density is reached. Consequently, they do not induce a humoral response in the human host. In contrast, non-*egc*-associated SAgS (e.g., *sea*, *seb*, *sec*, and *tsst-1*) are expressed in late-logarithmic and stationary growth, induce a specific antibody (Ab) response in the human host, and are a prominent cause of cause toxic shock (3).

SEB

Staphylococcal enterotoxin B (SEB) is the prototype of a non-*egc*-associated potent SAg. It is categorized as a category B select agent because it is the most potent staphylococcal enterotoxin, and much lower quantities are sufficient to produce a toxic effect than with synthetic chemicals. Furthermore, SEB is extremely stable and easily produced in large quantities. At low concentrations, SEB can cause multi-organ system failure and death. During the 1960s, when the United States had an offensive biological warfare program, SEB was studied as a biological weapon and stockpiled with various other bioweapons prior to its destruction in 1972 (4). Based on those investigations, the effective dose of SEB that would incapacitate 50% of the exposed population was estimated to be 0.0004 µg/kg of body weight, whereas the 50% lethal dose was estimated to be 0.02 µg/kg of body weight for humans exposed by the inhalation route. A convention on the “Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction” was signed by the United Kingdom, U.S., and U.S.S.R. governments in 1972. The U.S. government opted to reestablish research programs for vaccine and therapeutic development against biological weapons after suspicion arose that the U.S.S.R. was continuing the stockpiling and testing of biological weapons. Major NIH grant funding reinvigorated research on biological warfare agents after 11 September 2001 and especially after the anthrax attacks occurred in the U.S. mail system. Despite extensive efforts, however, there is no therapy or vaccine approved for human use against SEB to date.

THE INFECTION

Description of Agent

SEB is a well-characterized 28-kDa protein that consists of 239 amino acids and is most closely related to SEC1, with whom it shares structural as well as 67% amino acid homology (5, 6). SEB is water soluble, heat labile, and resistant to proteolytic enzymes, including pepsin, trypsin, and papain. The crystal structure of SEB was first determined in 1992 to a resolution of 2.5 Å (6) and later (7) to a resolution of 1.5 Å. The refined model contained 1,948 protein atoms and 177 water molecules and had an excellent geometry with root-mean-square (rms) deviation of 0.007 Å and 1.73° in bond lengths and bond angles, respectively. As a SAg, SEB cross-links Ag-presenting cells (APCs) and T cells by forming a ternary complex between the immune receptors major histocompatibility complex (MHC) class II and specific Vβ chains of the T-cell receptors (TcR) (6, 8, 9, 10). SEB protein is ellipsoidal, tightly and compactly folded into two unequal-sized domains of mixed α/β structure. Although the overall fold of SEB is similar to those of other microbial SAgS, it

lacks the zinc-binding site and only possesses one MHC class II binding site. The SEB residues implicated in TcR binding are 18, 19, 20, 22, 23, 26, 60, 90, 91, 177, 178, and 210. The suggested residues involved in binding of SEB to the MHC binding site are 43 to 47, 65, 67, 89, 92, 94, 96, 98, 115, 209, 211, and 215. Furthermore, the C-terminal disulfide loop (residues 113 to 126) in SEB has high flexibility, and it has been suggested to be responsible for emetic properties (6). Additional data from native gel electrophoresis and plasmon resonance affinity measurements indicate that the SEB-TcR complex can even form in the absence of MHC class II and that SEB-TcR interaction increases the binding to the MHC class II molecule DR1. It has been proposed that the finding that SEB can form complexes with TcR in both the absence and presence of MHC class II provides a mechanism for the ability of SEB to induce anergy in some cases and activation in others (11).

SEB is excreted by *S. aureus* strains from diverse clonal complexes. Most, if not all, staphylococcal strains designated as part of the CDC USA400 clonal group (by pulsed-field gel electrophoresis) produce large amounts of SEB or SEC. One study with isolates derived from New York identified SEB in four clonal complexes, with CC8 being the most common, followed by CC59, CC20, and one unassigned strain (12). Sequence analysis of 20 different *S. aureus* strains identified amino acid substitutions when compared to the SEB of strains COL and MNHO. These amino acid mutations involve positions 7 (lysine-asparagine), 14 (serine-alanine), 35 (alanine-serine), 125 (glutamine-histidine), 192 (asparagine-serine), and 222 (methionine-leucine) (13) (Fig. 1). It is noteworthy that these amino acid sequences lie outside the residues that are responsible for binding to MHC class II molecule and the TcR (Fig. 2). Investigations with purified, variant SEBs indicated that they varied in inducing proliferation of rabbit splenocytes in vitro as well as in lethality in a rabbit model of toxic shock syndrome (TSS) (13). Enzyme-linked immunosorbent assay (ELISA)-based quantification of SEB in supernatants of cultures in log phase demonstrates great variability among clinical *S. aureus* isolates, including sequential isolates derived from the same patient (12).

Interaction of Immune Cells with SEB

The primary targets of SEB are the TcR on T cells and the MHC class II molecules on APCs, resulting in a ternary complex between MHC class II molecules and specific V β chains of the TcR (6, 8, 9, 10) formed by this cross-linking. SEB binds to the MHC molecule outside the peptide-binding groove without prior processing, stimulating one of the seven Vh subclasses of the TcR (3, 12, 13.2, 14, 15, 17, or 20). Stimulated T cells then release large amounts of cytokines, namely interleukin-2 (IL-2), tumor necrosis factor alpha (TNF- α), and gamma interferon (IFN- γ), and undergo hyperproliferation and ultimately depletion. Cell adhesion molecules such as CD2 and ELAM on endothelial cells can also function as coreceptors for SEB-induced T-cell activation and cytokine production (14). The trimer complex activates intracellular signaling, which elicits phosphatidylinositol production and intracellular Ca²⁺ flux. This is followed by a rapid activation of membrane-associated protein tyrosine kinase and protein kinase C (15). Activation of the CD28-regulated signal transduction pathway is required for SAg stimulation in T cells and subsequent IL-2 production. Activation of transcriptional factors NF- κ B and AP-1 result in high-level expression of cytokines, including IL-1 and TNF- α from macrophages and TNF-

β , IL-2, and IFN- γ from T cells. Excreted cytokines have potent effects and cause fever, hypotension, multiorgan dysfunction, and ultimately, lethal shock. Table 1 summarizes the biological and pathological effects of SEB.

In vitro models to study SAg activity of SEB

In vitro cellular responses of SEB have been extensively studied in human peripheral blood mononuclear cells (PBMCs) and murine splenocytes. The MHC of murine cells has a lower affinity to SEB than the human HLA complex. Therefore, humans are many times more sensitive to SEB. Human PBMCs are sensitive to picomolar concentrations of SEB, whereas mouse splenocytes require nanomolar concentrations for stimulation. SEB-induced T-cell proliferation can be measured with different methods and usually peaks at 96 h in the employed in vitro systems. Most cytokine stimulation assays focus on quantification of IFN- γ and IL-2, but other cytokines are also induced, including IL-1, IL-6, IL-12, TNF- α , and IL-8. For optimal induction of proliferation, both T cells and monocytes are required. In vitro studies have demonstrated that SEB can also interact directly with TcR in the absence of MHC class II molecules, which results in an anergic T-cell response (11).

ANIMAL MODELS TO STUDY THE PATHOGENESIS OF SEB IN VIVO

Murine Models

Clearance of SEB has been investigated in mice after intravenous (i.v.) injection. SEB becomes systemically distributed within 5 to 30 min in blood and in lymph nodes (16). Clearance occurs within 10 to 24 h via glomerular filtration in the kidney. Small amounts of SEB are detectable also in the spleen. Manifestation of functional outcomes such as anergy, clonal expansion, and clonal deletion begins after 24 h.

Murine models with potentiating agents

Standard mice are not very sensitive to SEB due to low-affinity binding of SEB to murine MHC class II. Therefore, a potentiating agent is required to amplify the toxic effect of SEB. The list of potentiating agents includes lipopolysaccharide (LPS), D-galactosamine, and actinomycin D (10, 17). The hepatotoxin D-galactosamine induces TNF- α and produces fulminant liver failure and shock when given in combination with SEB, and higher levels of TNF- α are measured when compared to SEB alone. IL-2-deficient mice are more resistant to SEB-induced lethal shock (SEBILS), supporting the importance of IL-2 in the pathogenesis of SEBILS (18). The lethal shock in this model is associated with high concentrations of IL-1, IL-2, TNF- α , and INF- γ in the serum, which results in shock in mice. Studies that analyzed the cytokine level in the serum of mice treated with SEB or LPS alone or in combination (19) demonstrated higher TNF- α , IL-6, macrophage inflammatory protein 2, and monocyte chemoattractant protein 1 (MCP-1) levels in mice treated with both toxins than in those treated with either alone. Significantly higher levels of IFN- γ and IL-2 were observed at the later time point. However, it remains difficult to discern which cytokine induction is specifically caused by SEB in this model.

Murine models without potentiating agents

A dual-dose SEB model has been described for C3H/HeJ, a Toll-like receptor 4-defective mouse, and involves giving one dose of SEB (5 µg) administered intranasally followed by another dose of SEB (2 µg) intraperitoneally (i.p.) 2 h later, which results in high serum concentrations of IL-2, IL-6, and MCP-1 as well as elevated MCP-1 levels in the lung (20). The increased concentration of MCP-1, a potent activator and chemotactic factor for T cells and monocytes, may contribute to leukocyte recruitment into the lungs. Lethality and clinical signs of intoxication, such as ruffled fur and hypothermia, are similar to those observed in transgenic mice and nonhuman primates.

Another dual-dosing model for SEBILS is the HLA transgenic mouse model. These MHC class II knockout mice express the human MHC class II determinant DR3 in *trans* and are thus more sensitive to SEBILS. Two doses of SEB (50 µg) given 48 h apart i.p. induce toxic shock and 100% mortality (21). These mice also exhibit high levels of IFN-γ, IL-2, and IL-6 when SEB is administered by aerosol (22).

Rat Model

Rats have been used to study the effect of SEB in the central nervous system and to specifically clarify the role of the vagus nerve in sensation and transmission of abdominal SEB stimulation. In this model, i.p. administration of SEB (1 mg/kg of body weight) induced a robust Fos expression and induced activation of neurons in widespread brain areas, transmitting the signal of abdominal immune stimulation to the brain (23).

Rabbit Model

Rabbits are more sensitive to many staphylococcal toxins and develop pyrogenic symptoms similar to those of humans when SAgS are given by continuous perfusion (24). They also show a toxicity to SEB similar to those observed in staphylococcal food-borne illness, namely emesis when ingested orally, although this is not observed with the streptococcal SAgS or TSST-1. The role of SAgS (TSST-1, SEB, and SEC) in the setting of staphylococcal sepsis has been successfully investigated in a rabbit model of lethal pulmonary infection (25).

Piglet Model

A piglet model has also been used in assessing and understanding pathology and toxicity following native SEB challenge (26). For these models, weaning 7- to 14-day-old Yorkshire piglets are injected with SEB i.v. The clinical signs are biphasic, with pyrexia, vomiting, and diarrhea within 4 h, followed by terminal hypotension and shock by 96 h. Mild lymphoid lesions are identified as early as 24 h, with severe lymphadenopathy, splenomegaly, and prominent Peyer's patches by 72 h. Widespread edema—most prominent in the mesentery, between loops of spiral colon, and in retroperitoneal connective tissue—is found at 72 h. Additional histologic changes included perivascular aggregates of large lymphocytes variably present in the lung and brain, circulating lymphoblasts, and lymphocytic portal hepatitis. The piglet model has also been successfully used in oral vaccine studies. Like human cells, pig leukocytes readily respond to native SEB. Therefore, the piglet model is superior to mouse models which require potentiation of SEB toxicity (27) and the DR3

transgenic mouse model in mirroring the biphasic clinical response and overall pathology observed in humans. It is considerably cheaper than the rhesus macaque model.

Nonhuman Primate Model

In the late 1960s, rhesus monkeys (*Macaca mulatta*) were used to study SEB pathogenesis because they exhibit disease progression similar to that observed in humans (28). They manifest multiorgan failure and shock when injected i.v. Specifically, they exhibit acute renal failure, terminal depression of electroencephalographic patterns, fever, and emesis. A SEB-induced immediate-type skin reaction was also investigated in unsensitized monkeys. Substance P plays a predominant role in mediating intradermal SEB challenge and exerts its effect on cutaneous mast cells via stimulation of primary sensory neurons that contain substance P (29). The prohibitively high expenses and the limited number of monkeys that can be used per group limit the use of this model.

In summary, several animal models are available to test neutralization of SEB in vivo. It should be pointed out that SEB quantities differ between the models, as do the inherent sensitivities of the animals. This has to be taken into consideration when the efficacies of Abs are compared.

CLINICAL MANIFESTATION AND EPIDEMIOLOGY

SEB can cause several clinical symptoms in exposed humans. Manifestations of intoxication depend on the dose as well as the route of exposure.

Food Poisoning

SEB is one of the most common toxins implicated in toxin-mediated food-borne disease. Typically, heavily colonized food handlers contaminate food products with *S. aureus* via manual contact, coughing, or sneezing. *S. aureus* grows rapidly and excretes enterotoxins, especially in food products such as cream, mayonnaise, unrefrigerated meats, dairy, and bakery products. Heating the contaminated food only kills the bacteria but does not destroy the heat-stable, preformed SEB toxin. After ingestion of the toxin, the incubation period before patients become symptomatic is only approximately 4 to 6 h. This is also supported by data on occupational exposures in three laboratory workers at the U.S. Army Medical Research Institute who developed conjunctivitis with localized cutaneous swelling within 1 to 6 h, followed by gastrointestinal symptoms in two of the three workers after accidental cutaneous or ocular exposure to SEB (30).

Toxic Shock Syndrome (TSS)

TSS is characterized by the occurrence of fever, hypotension, multiple organ system dysfunction, rash, and desquamation, and it is classified as nonmenstrual or menstrual. The latter was first characterized in 1978 (31) in women that used tampons and is associated with TSST-1. The incidence has decreased significantly in past years (32), and nonmenstrual cases account for 55% of all cases (33). Those latter syndromes are commonly associated with SEB. TSS has been reported to occur in association with use of barrier contraceptives and after vaginal and cesarean delivery. It has also been reported in the setting of soft tissue

infection, endovascular infection, and visceral abscesses as well as upper and lower respiratory tract infection (34). Up to one-third of patients who have TSS develop recurrent disease. This requires persistent colonization with a toxigenic strain of *S. aureus* and only develops in patients who do not mount a humoral immune response to the implicated staphylococcal toxin (35). It is noteworthy that several case reports of staphylococcal TSS without rash have been described, which can make the diagnosis very difficult. It has been suggested that rash and desquamation result from delayed hypersensitivity, which is amplified by SAgS (36).

Atopic Dermatitis (AD)

AD is a common skin disorder that affects children during early childhood as well as adults. Patients with AD are frequently colonized with *S. aureus* strains. Comparison of colonizing *S. aureus* strains derived from patients with uncomplicated AD versus those derived from patients with chronic steroid-resistant AD indicate that the latter more commonly excrete SAg SEB. SAgS induce immunoglobulin E (IgE) Abs that are thought to exacerbate the skin and allergic inflammation in AD. Approximately 50 to 80% of patients with chronic AD have IgE Abs specific to SEA and SEB (37, 38). This hypothesis is further supported by data derived from a murine model of atopic dermatitis, where topical SAg exposure induces epidermal accumulation of CD8⁺ T cells, a mixed Th1/Th2 type dermatitis, and production of IgE Abs (39). A recent study observed predominance of SEB and SED in *S. aureus* isolates from AD patients with low IgE titers characterized by the prevalence of CD8⁺ lymphocytes and a dominant Th1 profile induced by SAgS and elevated IFN- γ expression (40).

Respiratory Diseases, Including Asthma and Nasal Polyps

Several studies suggest an association of colonization with SEB-excreting *S. aureus* and chronic rhinitis. A small clinical study comprised of 32 patients indicated a possible association between chronic rhinosinusitis and ulcerative colitis (UC). After functional endoscopic sinus surgery, the clinical symptom scores of chronic rhinosinusitis and UC severe scores were significantly reduced in these patients. Interestingly, the number of cultured *S. aureus* colonies from the surgically removed sinus mucosa significantly correlated with the decrease in UC severe scores, and high levels of SEB were detected in the sinus wash fluids of these patients.

In summary, SEB-mediated disease is diverse and extends beyond TSS. Recent studies strongly suggest that SEB excretion by colonizing strains may worsen inflammatory responses of allergic diseases.

DIAGNOSIS

SEB-mediated intoxication is usually diagnosed based on clinical suspicion and symptoms. The clinical signs of SEB intoxication are fever, vomiting, myalgia, diarrhea, headache, and in severe cases, lethal shock. Laboratory findings are not specific for the diagnosis of SEB intoxication, as nonspecific neutrophilic leukocytosis and an elevated erythrocyte sedimentation rate are present in many illnesses. A rising Ab titer response to SEB can be

helpful to validate the diagnosis retrospectively. Several methods to directly detect and quantify SEB have been developed in recent years. They include immunological assays such as immunodiffusion assays, radioimmunoassay, and ELISA, which have been applied for detection of SEB. Radioimmunoassay can detect up to 1 ng of SEB/ml in food extract; and ELISAs can detect less than 0.1 ng of SEB/ml in urine, blood, or food extract. Development of better instruments for mass spectrometry (MS) techniques has enhanced possibilities of more-precise structure identification and confirmation of proteins. Using this technique in combination with the Abs surface plasmon resonance chip has further enhanced the sensitivity and feasibility of these techniques. Currently, matrix-assisted laser desorption ionization–time of flight and electrospray ionization (ESI)–time of flight MS-based analysis can be completed within 1 h and have a very low detection limit of 3 pmol/ml of water (41). The combination of liquid chromatography-ESI MS/MS allows accurate determination by molecular mass and also by amino acid sequencing after enzymatic digestion. A sensitive laser nephelometric assay was developed to detect SEB in plasma of healthy volunteers as well as patients (42). Despite novel diagnostic approaches, evidence that SEB has actually been detected in human body fluids of either infected or intoxicated patients is scarce (43).

TREATMENT

There is no treatment available for SEB-mediated shock other than symptomatic support. The disease in the setting of food intoxication is usually self-limiting, and patients recover with active hydration and supportive measures. Steroids and antibiotics have not been shown to be effective for SEB intoxication (44). Many approaches to prophylaxis and therapy of SEB-mediated diseases have been explored and are outlined below. They include active immunization with inactivated recombinant SEB vaccines, synthetic peptides, and proteasome-SEB toxoid. Furthermore, Ab-based passive immunoprophylaxis/immunotherapy, as well as synthetic peptide antagonists and receptor mimics, such as chimeric mimics of MHC class II-TcR and of the TcR-V β , have been investigated. To date, the FDA has licensed no vaccine and antitoxin. However, numerous studies on various animal models for SEBILS have shown a favorable outcome with these diverse ranges of reagents that inhibit the proliferation of T cells and downregulate the expression of cytokine.

Peptide Antagonists

SEBILS can be successfully blocked with small overlapping antagonist peptides that inhibit the initial step of toxin-receptor interactions. Peptides directed to SEB amino acids 150 to 161 showed antagonist activity and protect mice from lethal shock against SEA, SEB, and TSST-1 when given i.v. 30 min after a lethal toxin dose (45). This conserved domain of SEB is not directly involved in MHC class II or TcR binding; however, it may be involved in interactions with coligands or cytotoxic T-lymphocyte antigen, which are necessary for superantigenic activity. A subsequent study also showed that peptides interfering with SEB domain residues 140 to 151 can block the proliferative effects against all staphylococcal and streptococcal SAgS and antipeptide Ab can protect passively against toxic shock in a rabbit model (46). However, a subsequent study indicated that these peptides were not effective in blocking T-cell activation, cytokine production, and SEB-induced toxic shock in HLA class

II transgenic mice as well as human T lymphocytes in vitro (47). Recently, another study demonstrated inhibition using dodecapeptide P72, which does not bind to MHC class II (48).

V β Domains

Another method to neutralize SEB action is by employing soluble forms of genetically engineered V β domains. These are high-affinity toxin-binding agents. A soluble G5-8 mouse V β (V β 8.2) mutant was generated and shown to be a promising therapeutic agent. Both administration of V β -TcR G5-8 as well as prior hyper-immunization to raise neutralizing Abs to SEB dramatically increase survival in a lethal pulmonary disease model in rabbits (49). Only equimolar amounts of these molecules are required to neutralize SEB, which indicates that they could have beneficial pharmacodynamic qualities.

Cytokine Inhibitors

Several drugs can interfere with cytokine induction and T-cell proliferation. They include the antibiotic doxycycline, which downregulates the SEB-induced proinflammatory cytokine and chemokine response as well as SEB-induced T-cell proliferation in human PBMCs (50). Furthermore, pentoxifylline, a methylxanthine derivative, and dexamethasone also inhibit SEB-induced activation of human PBMCs in vitro and also SEBILS in mice (51, 52). Recently, rapamycin, an immunosuppressant, has also been shown to inhibit cytokine release in vitro and toxin-mediated shock in mice (53). All of these therapeutic agents indirectly inhibit SEB-induced effects by downregulating the cytokine responses.

Immunotherapy

Von Behring and Kitasato first established immunotherapy by demonstrating that passive transfer of Abs from immunized animals could protect nonimmune animals against diphtheria. Before the discovery of sulfonamide in the 1930s, serum therapy was a common option to treat infectious diseases (54). Serum therapy remains the only prophylactic and therapeutic option against many toxin-mediated and viral diseases. Toxins are usually structurally distinct from the self-antigens expressed by the host cells and therefore safe targets for Ab therapy. One problem is that most investigators only screened monoclonal Abs (MAbs) alone and characterized them as protective, indifferent, and disease enhancing. This approach neglects the fact that naturally occurring Ab responses are complex polyclonal mixtures of Abs. However, in part, this simplified approach is chosen because the interaction between multiple Abs and toxins is complex and not easily predictable, let alone reproducible. Thus, FDA approval would be more difficult to obtain for mixtures of Abs. Currently, 39 MAbs are licensed by the FDA for human use for diverse indications, in addition to several polyclonal sera from diverse sources. Based on decades of successful use, Abs are considered valuable candidates for novel drug development because of their unique pharmacological qualities and safety profiles.

Epidemiological and Clinical Evidence

Since 11 September 2001, the development of Abs to neutralize toxins that could potentially be used in biological warfare has substantially increased, specifically for toxins like ricin, anthrax, Shiga toxin, pertussis, and SEB (55). For SEB, this research is highly justified

because both clinical and experimental data strongly support the concept that immunoglobulins can be used to treat SEB-mediated disease. SEBILS in animals and humans involve induction of several proinflammatory cytokines, including, e.g., TNF- α . Passive immunization with neutralizing anti-TNF- α MAb can prevent SEB-induced lethality. Although this establishes for TNF- α a pivotal role in SEB-mediated disease (10), this MAb does not neutralize SEB but only the effects of SEB induction. Similarly, Abs to costimulatory molecules, like anti-B7.2 MAbs, significantly inhibited T-cell activation by lowering systemic IL-2 release, blastogenesis, and IL-2 receptor expression and thus improved SEBILS survival in mice (56). Specific therapy, however, would be preferable, as it prevents rather than disrupts the cytokine induction.

Epidemiological data is consistent with the notion that Abs matter because older patients and healthy blood donors are more likely to exhibit Abs against SEB- and TSST-1-induced shock and recurrence is more common in younger patients who do not have Abs (34). Titers in patients vary and predict susceptibility to presumed toxin-mediated disease. In addition, investigations with serum from healthy blood donors demonstrated that immunoglobulin counteracted SEB stimulation in T-cell assays (57).

Vaccine Data

Several SEB vaccines have been tested in the past. The U.S. Army Medical Research Institute first started but ultimately abandoned the development of a vaccine to SEB using formalin-inactivated SEB toxin. SEB toxoid can be generated by prolonged incubation of SEB in formalin at pH 7.5. Despite retained immunogenicity and its ability to induce protective Abs in monkeys and rabbits, repeated oral doses of SEB toxoid proved to be poor mucosal immunogens and were thus not efficacious against the enteric ill effects of orally given SEB. Later, SEB toxoids containing a nontoxic biodegradable adjuvant, poly (DL-lactide-co-glycolide) microspheres or proteasomes were shown to be capable of inducing long-lasting, high-titred Abs. This toxoid-elicited immunity promotes neutralization of toxin in vivo and aborts lethality in mice and rhesus monkeys (58, 59). Mucosal vaccination with attenuated recombinant SEB vaccine in conjunction with cholera toxin was explored in mice and nonhuman primate models and shown to be effective after challenge with wild-type SEB toxin (27, 60, 61). Mice immunized intranasally were fully protected against a lethal dose of wild-type SEB, whereas partial (75%) protection was seen when mice were immunized intragastrically. Site-directed mutagenesis of conserved receptor-binding surfaces of SEA and SEB has been employed to generate toxoids for vaccination. Key amino acid residues involved in binding to the TcR V β chain (N23) and MHC class II (F44) were substituted. Amino acid substitutions result in toxins with reduced SAg activity (61). A SEB triple mutant with three critical amino acid substitutions in the MHC class II binding portion (L45, Y89) and TcR V β chain-binding portion (Y94) also manifests reduced T-cell activation without altering the structure of the Ag (62). This toxoid was also explored as a vaccine candidate in piglets where it induced an adequate Ab response even without the addition of the cholera toxin adjuvant. In summary, results of vaccine studies underscore the importance of the humoral immune response and encourage efforts to generate Abs for passive immunotherapy (62).

Passive Immunotherapy

Murine MAb—Several SEB-specific murine MAbs have been described in the literature. However, the majority of them have not been rigorously tested in animal models, and thus, their ultimate neutralization capacity is difficult to judge (21,56, 63, 64). Table 2 summarizes the list of MAbs generated against SEB toxin.

Four murine MAbs, B334 (IgG1), B327 (IgG2b), B87 (IgG1), and 2B33 (IgG1), which exhibit nanomolar range affinity have been described, and they recognized different, nonoverlapping epitopes to SEB (64). Of those, B87 and 2B33 inhibited the KS-6.1 (V β 8.2) T-cell response to SEB. To our knowledge, these MAbs have not been further tested in in vivo models.

A neutralizing murine anti-TSST-1 MAb (MAb5) which cross-reacts with SEB was evaluated for neutralization of SEB-induced superantigenic activities in vitro (63). The Ab was found to partially inhibit SEB-induced T-cell mitogenesis (63%) and TNF secretion (70%) in human PBMCs. Epitope mapping revealed that this Ab bound to TSST-1 residues 47 to 56 (₄₇FPSPYYSPAF₅₆) and to SEB residues 83 to 92 (₈₃DVFGANYYYQ₉₂), sequences that are structurally dissimilar. These studies were also not analyzed further in in vivo models.

Recently, four murine MAbs (20B1, 14G8, 6D3, and 4C7) specific to SEB were investigated, and three of four MAbs showed significant inhibition of SEB-induced T-cell proliferation as well as IL-2 and IFN- γ production by human T cells in vitro (21). These MAbs bind to different conformational epitopes that are destroyed by deletion of the distal C terminus of SEB. In spite of inhibition of T-cell proliferation in vitro, these MAbs differed in protective efficacy in a SEBILS mouse model. MAbs 14G8 and 4C7 were not effective in in vivo BALB/c and HLA-DR3 mouse models. MAb 20B1 was 100% protective in both mouse models, whereas 6D3 was partially protective in the BALB/c model but nonprotective in the HLA-DR3 model. In addition, enhanced protection against SEBILS was demonstrated when two nonprotective MAbs, such as 14G8 and 6D3, were combined in vivo even if they were less protective or nonprotective in monotherapy in the HLA-DR3 model. This study was important, as it demonstrated the superiority of combination therapy, possibly because of altered toxin clearance via Fc receptor-mediated uptake.

The SEB-neutralizing MAb-20B1 has also been shown to be an effective treatment in methicillin-resistant *S. aureus* infection in three mouse models. Administration of mAb-20B1 protects mice from lethal sepsis and reduces invasion of skin tissue and deep abscess formation. This study demonstrates further evidence for the role of SEB in *S. aureus* infections and a rationale for anti-SEB IgG as an immunotherapeutic agent for treatment of severe staphylococcal infections (65).

Chicken—SEB-specific Abs generated in chickens (IgY) successfully inhibited SEB-induced T-cell proliferation and cytokine responses in vitro and in passive transfer-protected mice. Rhesus monkeys were also protected from lethal SEB aerosol exposure when treated with the IgY specific for SEB up to 4 h after challenge. The advantage of chicken Abs would be substantial cost savings (66).

Chimeric and human Abs—In the more recent era of Ab development, construction of chimeric and humanized Abs have been aggressively pursued, as they can be expected to be less immunogenic and can potentially also confer human constant region function. A group of investigators generated chimeric human-mouse SEB-specific Abs (67). Two good candidates were identified and further investigated. At all SEB concentrations, significant neutralization of SEB-induced T-cell proliferation (human and mouse) was achieved with the chimeric Abs Ch82M and Ch63. Interestingly, improved neutralization between the combination of anti-SEBs and either Ab used alone was also noted. These chimeric Abs manifested affinities in the picomolar range. The chimeric Abs have also been tested in HLA-DR3 transgenic mice and achieved partial protection with one and complete protection with a combination of MAbs. In addition, these chimeric Abs were also shown to be effective both in vitro and in vivo when combined with lovastatin (68).

More-recent work has focused on developing human MAbs to SEB. The inhibitory and biophysical properties of 10 human Fabs, derived by panning after vaccination with STEBVax, were examined. These Fabs exhibited binding affinities equal to polyclonal IgG, had low-nanomolar 50% inhibitory concentrations against SEB in cell culture assays, and partially protected mice from SEBILS. This study used an LPS-potentiated model with fairly low doses of SEB (2.5 µg). Fabs also bound to SEC1 and SEC2 as well as streptococcal pyrogenic exotoxin C. Four Fabs against SEB, with the lowest 50% inhibitory concentrations, were converted into native full-length MAbs. Of note is that a 250-fold-greater inhibition of SEB-induced T-cell activation was observed with two MAbs than with their respective Fab fragments, which had equal binding affinities (69). SEB-specific fully human MAbs were also generated using the “human MORPHO-DOMA technology” after isolating B cells from healthy donors whose sera showed preexisting high immune reactivity to SEB. Human MAb 154 showed an inhibitory effect on SEB-induced secretion of proinflammatory cytokines specifically tested for IFN-γ and TNF-α by human PBMCs and protected mice prophylactically from a challenge of up to 100 µg of SEB injected i.p. potentiated by LPS (70).

In addition, using phage display technology, human Ag-binding fragments have been synthesized and converted into fully human IgG Ab. These synthetic human MAbs display affinities in the nanomolar range. They were effective at a dose of 200 µg in the murine SEBILS model using different challenge doses of SEB (71).

FUTURE CHALLENGE

Current data from various studies are encouraging and predict that neutralization of SAGs like SEB is feasible. Future developments should focus on developing high-affinity MAbs that exhibit good pharmacokinetic parameters and will permit treatment with lower doses. Several studies indicate that combination of MAbs may result in more potent neutralization. A major challenge is to define an adequate setting in which these Abs can be tested, as patients with toxic shock are difficult to identify, let alone provide consent for a study. The FDA has recently approved raxibacumab to treat inhalational anthrax. This is the first MAb approved using the animal efficacy rule. So one option would be to test these MAbs only in nonhuman primate models, which is possible under the new FDA animal efficacy rule.

Another option would be to better define the SEB-induced allergic syndromes that could constitute human patients to test the effect of neutralizing Abs. Another challenge will be to identify the selective pressures on bacteria expressing SAgS and how the selective pressures influence the interaction between SAg-bearing bacteria and the human immune system. This knowledge may prove invaluable to prevent emerging diseases mediated by SAgS and, most importantly, to improve the management of SAg-associated diseases. In this regard, care will have to be taken to monitor for the emergence of SEB variants that may not be effectively neutralized by all Abs.

ACKNOWLEDGMENTS

We thank Kaushik Dutta for generating the figure.

BCF was supported by NIH grant U54-AI057158 and in part by an award from Pfizer's Centers for Therapeutic Innovation, New York.

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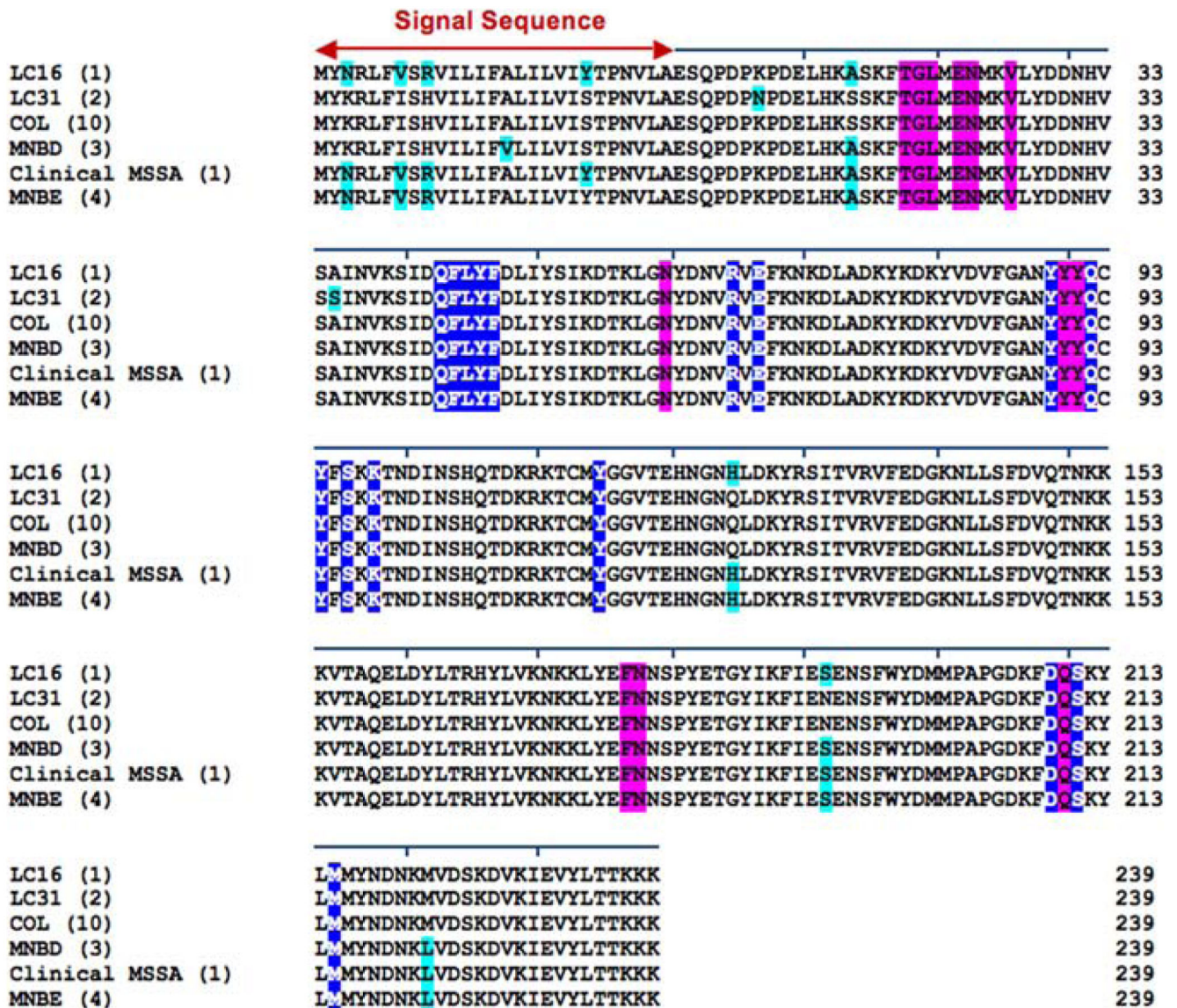
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**FIGURE 1.**

Alignment of amino acid sequences of SEB derived from *S. aureus* clinical isolates. Amino acid mutations are highlighted in green. MHC- and TcR-interacting residues are shown in blue and magenta, respectively. doi:10.1128/microbiolspec.AID-0002-2012.f1

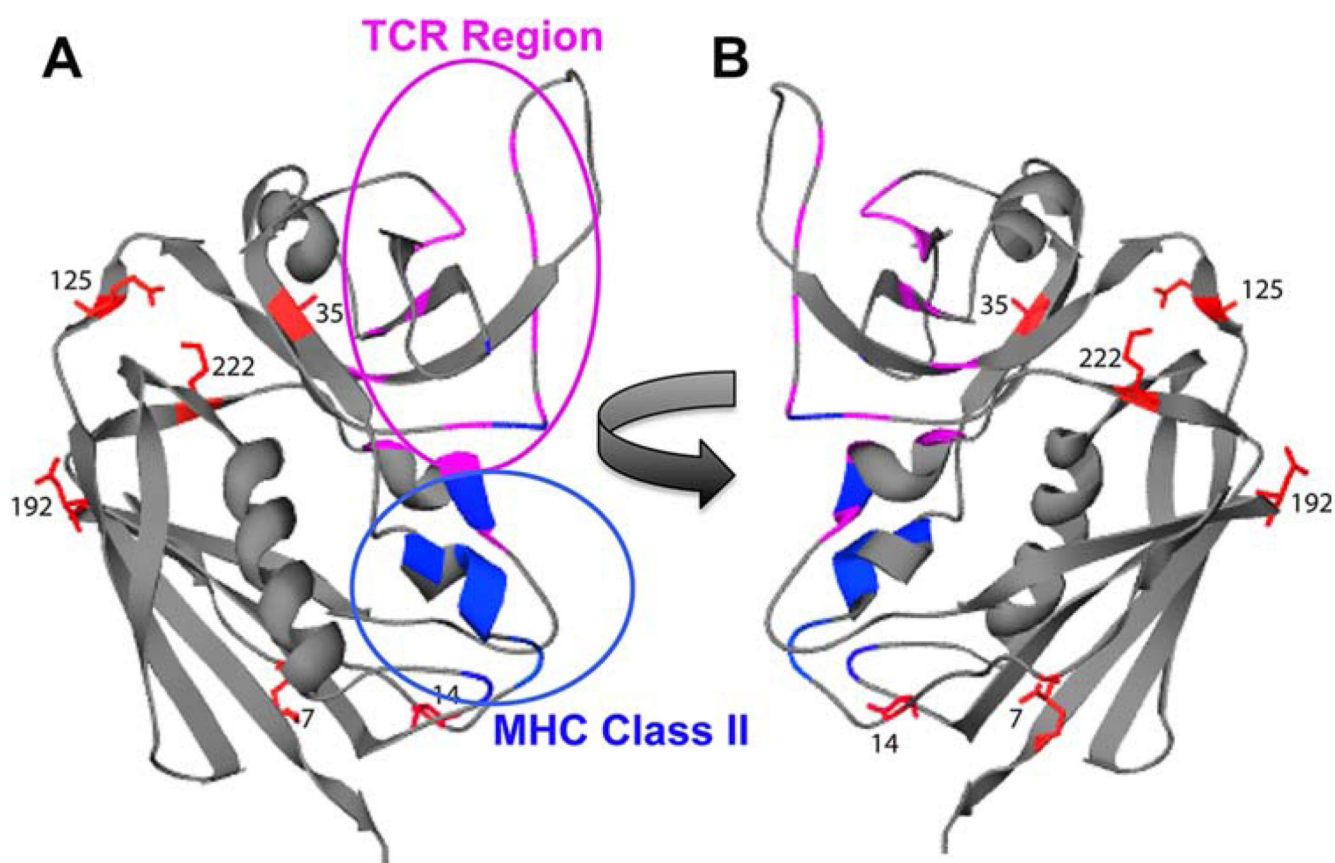


FIGURE 2.

(A) Ribbon structure of SEB protein showing amino acid mutations in *S. aureus* isolates. Residues which interact with MHC and TcR are shown in blue and magenta, respectively.

(B) View after rotating 180 degrees around vertical axis. doi:10.1128/microbiolspec.AID-0002-2012.f2

TABLE 1

Major biological and pathological activities of SEB

Superantigenicity: proliferation of CD4 T cells following binding with the V β motif of TcR and MHC class II molecules on the surface of APCs
Induction and release of several cytokines
Lethality and shock in experimental animals, including mice, rabbits, piglets, and monkeys
Emetic activity
Direct or indirect involvement in pathogenesis of severe diseases, including TSS, nonmenstrual TSS, atopic dermatitis, asthma, and chronic rhinitis

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TABLE 2

List of MAbs generated against SEB toxins

Source	Ab(s)	Affinity unit(s)	In vitro model(s)	In vivo model(s)	Reference
Murine	IFD7, 2DA3 and 2HA10, 2EG5 and 2GD9		BALB/c splenocytes		72
Murine	B334, B327, B87, 2B33	nM	Mouse V β 8.2 + T cell (KS-6.1)		64
Murine	MAb5 (anti-TSST MAb)		Human PBMCs		63
Chicken	IgY		BALB/c	Rhesus monkeys	66
Murine phage display	Soluble SEB-ScFv	nM			73
Chimeric	Ch82M and Ch63	pM	HLA-DR3 mouse splenocytes		67
Human	10 Fab and 4 full-length MAbs	nM	Human PBMCs	BALB/c	69
Human	Human MAb79G9, human MAb154	pM–nM	Human PBMCs	BALB/c	70
Murine	20B1, 14G8, 6D3, 4C7		Human T cells	BALB/c, HLA-DR3	21
Chimeric + lovastatin	Ch82M and Ch63 with lovastatin		BALB/c mouse splenocytes	HLA-DR3	68
Murine	3F3 (anti-SEAand -SEB)				74
Synthetic human MAb	IgG 075, IgG 079, IgG 079-P, IgG 119, IgG 120, IgG 121	nM	Human PBMCs	BALB/c	71