3

Staphylococcal Enterotoxins, Stayphylococcal Enterotoxin B and Bioterrorism

Martha L. Hale
United States Army Research Institute of Infectious Diseases, Integrative Toxicology Division, Fort Detrick, USA

1. Introduction

Staphylococcal enterotoxins (SEs) are exotoxins produced primarily by *Staphylococcus aureus,* which is a ubiquitous microorganism with world-wide distribution (Bergdoll, 1983; Dinges et al., 2000). SEs are a major cause of food poisoning and they are also potent immune activators that lead to serious immune dysfunction (Alouf and Muller-Alouf, 2003; McCormick et al., 2001). Unlike most toxins, SEs are not directly cytotoxic and cell entry is not a requirement for them to cause an effect. The Centers for Disease Control and Prevention (CDC) place one SE, staphylococcal enterotoxin B (SEB), as a select agent based on its universal availability, ease of production and dissemination, and the potential to cause moderate but widespread illnesses. Additionally, because these agents are common to the environment and the diseases they cause are similar to other diseases, Category B agents require close environmental monitoring and enhanced disease surveillance (http://www.bt.cdc.gov/bioterrorism/).

Many biothreat agents are common inhabitants of the soil and animals, and are known to cause disease in areas where they are indigenous. SEs present an additional problem in that SE-producing *S. aureus* are found throughout the world, and are known to produce a variety of illnesses, so that detection of a possible bioterrorist attack may be more problematic than those of other agents (Ahanotou, et al., 2006). The following sections describe SEB's history as a biowarfare agent and its possible use as a bioterrorism agent. To understand why it is considered a Category B agent, a description of the toxin, the main diseases caused, methods to treat the diseases, and surveillance mechanisms will also be discussed.

2. Biowarfare history

In the era of offensive biological weapons, one of the SEs, SEB, was studied, not so much for its mass destruction capabilities but, rather for its ability to incapacitate soldiers so they would be incapable of fighting or defending their posts (Croddy and Hart, 2002; Hursh et al., 1995). The United States bioweapons program studied the toxin intensively and determined that the amount of SEB required to induce incapacitation was considerably less than that of synthesized chemicals. When the toxin and chemicals were compared by expense, time, and complexity of production, SEB was far more cost-effective. A dose of 400 pg/kg body weights was estimated to incapacitate 50% of the human population exposed

by an aerosol attack, while 200 ng/kg body weights would be lethal for 50% of those exposed (Ahanotu, et al., 2006; Bellamy and Freedman, 2001; Ulrich et al., 1997).

By 1966, the U.S. and its allies had produced stockpiles of various biowarfare (BW) agents, including SEB (under the code name WG) and research to establish parameters for SEB's use as an aerosolized bioweapon continued at several facilities in the U.S. and Great Britain. In the fall of 1969, President Nixon stopped the offensive BW program and by 1972, all stockpiles of agents were destroyed (Greenfield et al., 2002; Franz et al., 1997). On April 10, 1972, Great Britain, United States, and Soviet governments signed the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, which went into effect March 26, 1975.

During the latter half of the Cold War, the Defense Intelligence Agency (DIA) and the Central Intelligence Agency (CIA) suspected that the USSR was continuing to stockpile and test biological weapons and therefore, defensive research programs were established for vaccine and therapeutic development (Ulrich et al., 1997). Not only have these research programs aided in development of surveillance mechanisms, the programs have significantly contributed to a greater understanding of diseases and the development of possible therapeutic interventions.

With the end of the Cold War and dissolution of the USSR, threat of BW was greatly diminished. Other rogue nations were still stockpiling weapons and the CIA uncovered evidence that Iraq was building an arsenal of biological weapons. Although weaponized SEB was considered a high probability, it was not found when the Iraqi weapons program was dismantled (Zalinskas, 1997).

3. The toxin

SEB is one of several exotoxins isolated from *S. aureus* that are known for their emetic and superantigen traits (Bergdoll et al., 1974; McCormick et al. 2001). These exotoxins were the first superantigens to be identified, but since their discovery, additional superantigens have been isolated in other bacteria, particularly from the closely related genus, *Streptococcus*. Although staphylococcal and streptococcal superantigens are very similar, descriptions of toxin here will be limited to those toxins produced by staphylococci and the toxins will be identified as SEs or superantigens (SAG).

3.1 Description of the toxin

SEB belongs to a group of pyrogenic enterotoxins, produced primarily by *S. aureus* (McCormick et al., 2001). They are water soluble and relatively resistant to heat and proteolytic enzymes, including pepsin, trypsin, and papain (Le Loir et al., 2003). Stability does also depend upon purity of the toxin preparation, the medium's composition, and the pH. SEB is one of the most stable toxins when exposed to extreme temperature and pH, one characteristic that makes SEB an attractive bioterrorism agent (da Cunha et al., 2007; Le Loir et al., 2003; Nout et al., 1988).

Although they vary in amino acid sequence, SEs share a common three-dimensional structure that maintains their unique binding regions (Fig. 1) (Baker and Acharya, 2004; Papageorgiou et al., 1998). At least 20 serologically distinct SEs have been isolated primarily

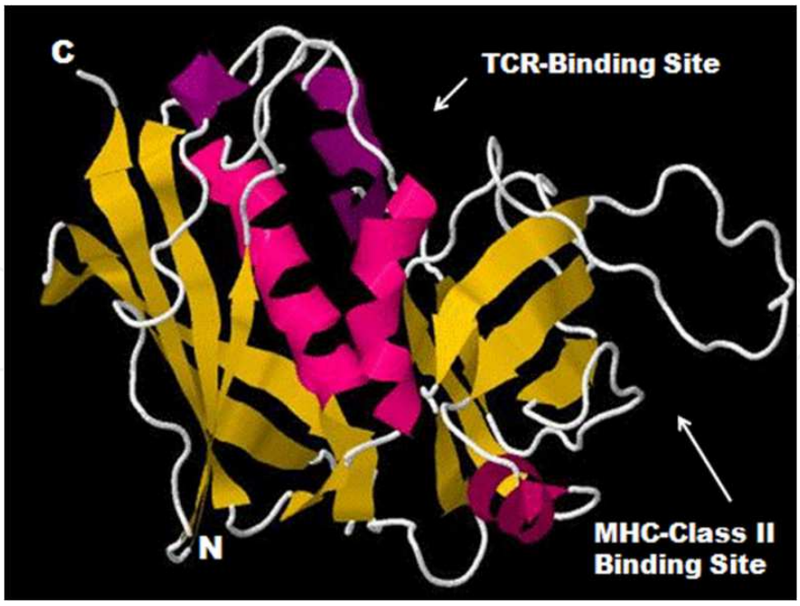

Fig. 1. Molecular structure of SEB (from PDB:3SEB using Jmol version 12.0.41) showing α-helices (magenta), β-strands (gold), and loop strucuture. The β-grasp domain is on the left, and the disulfide bond in the right. The N terminus and C terminus are labeled.

from *S. aureus* (Table 1). Using the Clustal W program, the amino acid sequences of the SEs were aligned and evolutionary distances determined. A dendrogram constructed by the near neighbor-joining method divides the toxins into three major and two minor monophyletic groups (Ono et al., 2008; Uchiyama et al., 2003). The first two groups contain the classical toxins SEA, SED, SEE (Group 1) and SEB, SEC (Group 2) in addition to newly identified SEs; Group 3 contains only newly identified toxins. There is some similarity in structure in that Groups 1, 2, and 5 have a disulfide bond while Group 3 and Group 4 (TSST-1) do not.

Many, but not all, SEs require zinc ions for functional binding to the MHC class II and for stability of its tertiary structure (Fraser et al., 1992; Ples et al., 2005); related to their amino acid sequences, the SEs bind zinc at various locations within the molecule (Brosnahan et al., 2010). Some bind zinc in the concave β sheet of the C terminal domain while others bind zinc in a cleft between the two domains. SEB and toxic shock syndrome toxin (TSST-1 do not bind zinc ions (Brosnahan and Shlievert, 2011; Ly, et al., 2001; Sundstrom, et al, 1996).

3.2 Genetic analysis of SE genes

Analysis of SE genes indicates divergence from a common ancestry. Most genes coding for the enterotoxins are found on mobile elements such as pathogenicity islands, plasmids and bacteriophages, making horizontal transfer a common occurrence (Jarraud et al., 2001; McCormick et al, 2001; Yarwood, et al., 2002). In 2001, a cluster of genes with homologies to SE genes was identified and named the enterotoxin gene cluster (egc). Since many of the genes produced SE-like proteins, Jarraud et al. (2001) suggested that the gene cluster formed an enterotoxin nursery where genomic rearrangements would lead to new SEs, a fact that has now been confirmed with the development of the new exotoxin SEG (Lindsay, 2011; Thomas et al., 2006).

Group[a]	Enerotoxin	MW (kDa)	Gene Location[b]	Emetic
1	SEA	27.1	phage	yes
	SED	26.9	plasmid (pIB485)	yes
	SEE	29.6	prophage	yes
	SEJ	31.2	plasmid (pIB485)	yes
	SEN	26.1	egc[c]	yes
	SEO	26.8	egc	yes
	SES	26.2	phage	yes
	SEP	26	phage	yes
	SHE[c]	25.1	transposon	yes
2	SEB	28.4	SaPI[d]	yes
	SEC1	27.5	SaPI	yes
	SEC2	27.6	SaPI	yes
	SEC3	27.6	SaPI	yes
	SEG	27	egc	yes
	SEIR	27	plasmid (pIB485)	yes
	SEU	27.2	egc	yes
3	SEI	24.9	egc	poor
	SEK	26	SaPI	yes
	SEL	26.8	SaPI	no
	SEM	24.8	egc	yes
	SEQ	26	SaPI	unknown
4	TSST-1	21.9	PI	no
5	SET	22.6	egc	poor

[a]Staphilococcal enterotoxins are divided into 5 monophyletic groups according to ammino acid sequence alignment (Uchiyama et al., 2003; Ono et al., 2008)
[b]Gene location of the toxin
[c]enterotoxin gene cluster
[d]Staphylococcus aureus phatogeniciti islands

Table 1. Staphylococcal enterotoxin/superantigens

As shown in Table 1, genetic elements containing SE genes vary. Most are located on mobile genetic elements (MGEs) which are DNA pieces with ends that encode genes (Lindsay, 2011). There are several types of MGEs, including plasmids, pathogenicity islands, bacteriophages, and transposons. MGEs move from one bacterium to another or between various genetic elements in the same bacterium. Mobility of the genes is thought to

contribute to the number and genetic variation within this group of toxins. Interestingly, however, diversity in amino acid sequences has not affected toxin binding to its receptors, suggesting that as the proteins evolved, selective pressures maintained their binding sites by keeping a tertiary structure that supports the characteristic binding (Baker and Achara, 2004; Ulrich et al., 2007).

Expression of SE genes is highly regulated by growth phase and environmental conditions, and not all conditions are suitable for gene activation (Lindsay, 2011). With the proper medium, most toxin production occurs in late log or stationary phase (Otero et al., 1990; Rahkovic et al., 2006; Soejima et al, 2007). *S. aureus* produces regulatory proteins and small RNAs that control toxin production, probably so that in harsh conditions, the bacterium can conserve energy (Horsburg, 2008; Fournier, 2008).

One major regulator of some, but not all SE toxin production, is the accessory regulator gene (Agr) system (Lindsay, 2011). When bacteria reach a critical mass, a quorum-sensing system activates Agr, which, in turn, activates some toxin genes (SEB, for example). Other regulatory systems such as SarA can also up-regulate toxin genes indicating that regulatory pathways are complex and multiple systems probably control toxin production.

3.3 Superantigen characteristics of SEB

Marrack and Kappler (1990) coined the term "superantigen" to connote the similarities between SEs and conventional protein antigens that activate T cells by cross-linking T cells to antigen-presenting cells (APC). Both superantigens (SAG) and conventional antigens bind to the major histocompatibility class II (MHC class II) receptor located on APCs (Haffner, et al., 1996). However, conventional antigens bind to MHC class II molecules inside their antigen-binding grove and are processed into peptides expressed on the cell surface before they are presented to T cells via the T-cell receptor (TCR). In contrast, SAGs bind directly to MHC class II molecules outside the antigen-binding groove; they are not processed into peptide fragments before presentation to TCRs (Fig. 2).

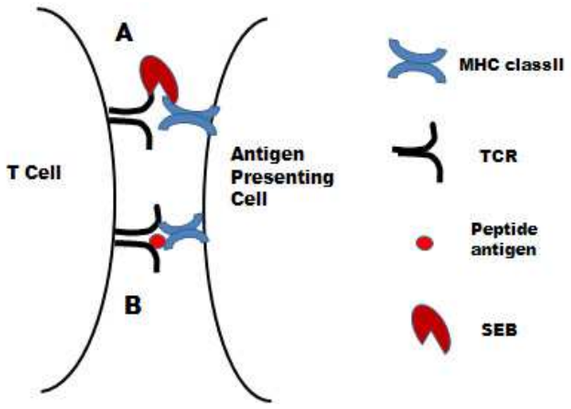

Fig. 2. Conventional antigen and SAGs bind APC and TCR. (A) SAGS bind to the outer region of the TCR Vβ and to the outer region of the MHC Class II determinant. (B) Conventional antigens are processed into peptides that are then presented to the antigen-binding region of the TCR.

While SAG must first bind to MHC class II molecules, their ability to activate large populations of T cells depends largely upon their binding to the TCR (Alber et al., 1990; Pontzer, et al., 199; Stevens, 1997; White et al., 1989). There are five different elements (Vα, Jα, Vβ, Dβ, and Jβ) that comprise the two-chain TCR receptor with each composed of a constant and variable region. The TCR uses all five elements for recognition of processed peptides on the surface of APCs (from conventional antigens). Peptide binding occurs within the peptide-binding region and requires a helper molecule such as CD4. Superantigens bind only to Vβ elements and specificity of binding results from the types of Vβ molecules present (Fig. 1). For example, SEB binds to human Vβ phenotypes 3, 12, 14, 15, 17, which differ from phenotypes bound by other SAGs (Table 2). Because many T cells contain the same Vβ phenotype, SAGs may activate up to 20% of the whole T-cell population rather than the 0.01% activation by conventional antigens.

SAGs are not restricted and can bind to both CD4 and CD8 T cells if the SAG recognizes the TCR Vβ chains, which increases the number of T cells they can affect. Superantigen-activated T cells can undergo at least five to six rounds of cell division (Nagshima et al., 2004). The massive clonal T-cell expansion results in the activation of programmed cell death in which cells responding to the specific superantigen are deleted (Choi et al., 1989; Yuh et al., 1993). Apoptosis of large numbers of T cells, clonal deletion and anergy of specific T-cell populations, and massive release of proinflammatory cytokines are all factors in the toxin's pathogenesis.

4. SE pathogenesis

There are two major diseases caused by the staphylococcal enterotoxin superantigens (SEs), and most diseases attributed to the SEs relate to chronic disease states caused by autoimmunity and repetitious stimulation by SEs. Those autoimmune diseases in which SEs are thought to play a role require more than one challenge and therefore, will probably not be a concern in a bioterrorism threat. The two diseases that are pertinent to potential bioterrorism attacks are SEB (or SEs) in the food or water supply (food poisoning) or an aerosol attack in which the toxin will be inhaled into the lungs, possibly causing toxic shock syndrome.

4.1 Food poisoning

As noted previously, *S. aureus* is a ubiquitous microorganism with a world-wide distribution, and is responsible for causing large numbers of food poisoning cases throughout the world (Bergdoll, 1989; Le Loir et al., 2003; Ortega, et al., 2010). In its normal environment, the gram-positive cocci and its toxins do not cause disease; however, when introduced into foods such as cream, mayonnaise, or similar foods, the bacteria grow rapidly secreting the exotoxins which then contaminate the food. Dack and coworkers (1930) provided the first documented report that identified a toxin from *S. aureus* as a causative agent of a food poisoning incident involving staphylococci-contaminated Christmas cake. The investigators grew the bacteria isolated from the cake and found that a sterile filtrate from the broth in which the bacteria were grown induced food poisoning when ingested by human volunteers. Thereafter, from investigations of various food poisoning outbreaks, an initial five antigenically distinct enterotoxins were identified suggesting that *S aureus* produced a family of protein toxins possessing similar properties and virulence (Casman,

1960; Bergdoll et al., 1959; Bergdoll et al., 1965; Casman et al., 1967). Since the characterization of the five serotypes, at least 20 more SEs (Table 1) have been isolated and characterized with many inducing emesis in monkeys or humans (Uchiyama et al., 2003).

Enerotoxin	TCR Vβ Specificity
SEA	1.1,5.3,6.3,6.4,6.9, 7.3,7.4,9.1,18
SEB	3,12,14,15,17,20
SEC1	3,6.4,6.9,12,13.2,14,15,17,20
SEC2	12,13.2,14,15,17,20
SEC3	3,5,12,13.1,13.2
SED	5,12
SEE	5.1,6.3,6.4,6,9,8.1,18
SEG	13.6,14,15
SHE	Vα10
SEI	1,5.1,5.2,5.3,23
SEJ	ND
SEK	5.1,5.2,6.7
SEL	5.1,5.2,6.7,16,22
SEM	18,21.3
SEN	9
SEO	5.1,7,22
SEIP	5.1,6,8,16,18,21.3
SEQ	2,5.1,5.2,6.7,21.3
SEIR	3,11,12,13.2,14
SEU	13.2,14
TSST-1	2,4

Table 2. Staphylococcal enterotoxins showing the corresponding TCR Vβ repertoires

Approximately 25% of healthy people and animals carry *S. aureus* on the skin and often food workers who carry the bacterium may contaminate food when they handle food without washing their hands or wearing gloves. The microorganism is also found in unpasteurized milk and cheese products and, being salt tolerant, grows in salty foods as well. Because they are highly resistant to heat and enzymatic inactivation, foods that do not require cooking or those prepared by hand provide greater risks of contamination with the bacteria and subsequent toxin production. The short incubation period, approximately 4-6 hr after ingestion, usually differentiates SE-induced food poisoning from those caused by bacteria such as *E. coli* or *Salmonella* species where presence of the bacteria is required for disease.

The onset is sudden and vomiting is the hallmark symptom. Other symptoms, such as diarrhea, abdominal pain, and nausea may also be present, but systemic manifestations such as fever are very uncommon (Alouf and Muller-Alouf, 2003; Kerouanton et al., 2007). Although extremely incapacitating, staphylococcal food poisoning is usually self-limiting and symptoms last about 1 day. Fatality in healthy adults is rare (0.03%); however, the rate is higher in susceptible populations (children, elderly, and immune-compromised adults) and may also depend upon the concentration of the toxin ingested (Do Carmo, et al., 2004).

SE-induced food poisoning was initially thought to be caused by the local interaction of the toxin with intestinal cells because the toxin stimulates nerve centers in the gut through serotonin (5-hydroxytryptamine or 5 HT) release from intestinal mast cells (Alouf and Muller-Alouf, 2003; Hu et al., 2007). Serotonin binds to 5-HT_3 receptors which are ligand-gated ion channels located on the afferent vagus nerve terminals. The binding of serotonin to the receptor opens the channel which signals the medulla emetic reflex center to generate nausea and an emetic response. However, such interactions do not explain the disease pathophysiology. Patients with SE intoxication can exhibit rather severe gastrointestinal damage including mucosal hyperemia, regional edema, petechiae, and purulent exudates (Ortega et al., 2010; Palmer, 1951). SEs cross the intestinal epithelial barrier and gain access to local and systemic lymphoid tissues, suggesting that activation of local immune tissue may be partially responsible for gastrointestinal damage (Hamad et al., 1997). Involvement of the immune system in pathogenesis could explain why immuno-compromised adults develop a more severe, life-threatening disease than normal healthy adults. However, emesis is not directly linked to T-cell proliferation because TSST-1, a potent immune activator, does not cause emesis. TSST-1 is more susceptible to enzymes in the digestive tract and could be the reason for its lack of emetic activity. SEs with emetic activity have a disulfide bond located at the top of the B domain and probably are responsible for stabilizing the molecule in a conformation needed to induce emesis (Brosnahan and Schievert, 2011).

4.2 Toxic shock syndrome

During the late 1970's, Todd and coworkers (1978) described an acute illness in seven children, between the ages of 8 to 17. Symptoms included high fever, hypotension, vomiting, watery diarrhea, a scarlatiniform rash, and renal failure. Although bacteria were not isolated from blood, cerebrospinal fluid, or urine, S. aureus was isolated from mucosal sites. Culture filtrates from cultures of these isolates were shown to contain a toxin that would cause a rash (Nikolksy sign) in newborn mice. Todd named the new disease toxic shock syndrome (TSS). In 1977 through 1980, 22 women between the ages of 13-24, were diagnosed with TSS (Chesney et al., 1981). Investigations showed that this TSS was the result of highly absorbent tampons, which became contaminated with *S. aureus*. A toxin, tsst-1, was isolated from the bacteria cultured from the contaminated tampon and shown to be similar to that discovered by Todd et al. (1978). The super absorbent tampons were found to introduce oxygen into the anaerobic environment of the vagina which facilitated the growth of *S. aureus* and release of TSST-1. When the absorbent tampons were taken off the market, the incidence of menstrual TSS decreased dramatically.

From these early investigations, TSS has been divided into two categories. The first, menstrual TSS, occurs primarily in young women, ages 16-25, during menstrual periods and is usually associated with tampon use. As first identified in the 1980s, TSST-1, remains the

leading cause of menstrual TSS (Chesney et al, 1981; Brosnanhan and Schlievert, 2010). TSST-1 is also a major etiologic agent for the other TSS category, non-menstrual TSS, comprising 50% of the reported cases (Buchdahl et al, 1985). Two other SEs, SEB and SEC, have been identified as the causative agent in most of the remaining 50%. Since its discovery, TSS had been considered a rare, but often fatal disease. After removal of absorbent tampons from the market and efforts to inform the public about TSS, incidences of menstrual TSS dropped from 13.7% to 0.3% per 100,000 individuals in the U. S. Since 1986, reported incidences of TSS in the U.S. have remained stable with the annual incidence rate around 0.32%-0.52% per 100,000 people (DeVries, et al., 2011; Haijeh et al., 1996). Several reasons may account for the disease's rarity. One reason lies in the fact that most adults have been exposed to SEs over a period of many years, and therefore, possesses antibodies against many of the SEs. The lack of anti-SE antibodies could also explain why children and young adults are more susceptible to the disease. In addition to immunity against SEs, another problem in TSS diagnosis relates to the lack of a single diagnostic test and, therefore, diagnosis relies upon a complex analysis of clinical symptoms. Requirements for a case to be identified as TSS are rigorous, and while SEs are significant virulence factors in many infections, most infections do not meet the diagnostic criteria for TSS as established by CDC (Table 3).

TSS pathophysiology involves many intricate extracellular and intracellular signaling pathways and at this time, the exact pathways or pathways responsible for the syndrome are not known (Davis et al., 1980; Kumar et al., 2010; Pinchuk et al., 2010). Hallmark studies during the 1990s showed that the toxicity of SEB was due to massive T-cell proliferation and proinflammatory cytokine production (Marrack et al., 1990; White et al., 1989). Investigations using T-cell reconstituted immuno-deficient SCID mice confirmed the role of T-cell activation in SE-induced lethality (Miethke et al., 1992; Canaan, et al. 1999). Furthermore, these studies indicated that tumor necrosis factor alpha (TNF-α) plays a crucial role in SE-induced lethality because passive immunization with an anti-TNF-α/β monoclonal antibody protected the animals against an SE challenge (Fast et al., 1989; Miethke et al., 1992). Although the precise mechanisms by which proinflammatory cytokines induce TSS, these early studies and further investigations provide overwhelming evidence that tissue damage, shock, and multiple organ failure is caused by the production of pathological concentrations of proinflammatory cytokines and chemokines such as TNF-α, interleukin 1β (IL-1 β), interferon gamma (IFN-γ) and interleukin 6 (IL-6), and macrophage chemoattractant protein-1 (MCP-1) (Marrack et al., 1990; Williams, 1991).

Although T cells appear to play a dominant role in TSS, more recent investigations indicate that SE interactions with other cell types may also contribute to TSS pathophysiology (Das, 2000; Faulkner et al., 1997; Marrack et al., 1990; McCormick et al. 2001). Initially, the purpose of the APC MHC class II interaction with SEs was thought to provide a mechanism for T-cell activation and production of proinflammatory cytokines by T-cell populations. That MHC class II interactions play a more active role in TSS was shown by studies in which mortality from TSST-1 was not reduced in T-cell depleted rabbits (Dinges et al, 2003). In addition, TSST-1 induced proinflammatory cytokines in these animals, again suggesting that other cells may play a role in cytokine production. These studies are further supported by investigations in which the SE-MHC class II interaction by itself was shown to be sufficient to activate intracellular signaling pathways which induce downstream pro-inflammatory signaling and subsequent production of cytokines (Kisner (a) et al., 2011; Kisner (b) et al., 2011).

Clinical case definition

- Fever: temperature greater than or equal to 38.9°C
- Rash: diffuse macular erythroderma
- Desquamation: 1-2 weeks after onset of rash
- Hypotension: systolic blood pressure less than or equal to 90 mm Hg for adults or less than fifth percentile by age for children aged less than 16 years

Multisystem involvement (three or more of the following organ systems:

- Gastrointestinal: vomiting or diarrhea at onset of illness
- Muscular: severe myalgia or creatine phosphokinase level at least twice the upper limit of normal
- Mucous membrane: vaginal, oropharyngeal, or conjunctival hyperemia
- Renal: blood urea nitrogen or creatinine at least twice the upper limit of normal for laboratory or tract infection
- Urinary sediment with pyuria (greater than or equal to 5 leukocytes per high-power field) in the absence of urinary tract infections
- Hepatic: total bilirubin, alanine aminotransferase enzyme, or asparate aminotransferase enzyme levels at least twice the upper limit of normal values
- Hematologic: platelets less than 100,000/mm3
- Central nervous system: disorientation or alterations in consciousness without focal neurologic signs when fever and hypotension are absent

Laboratory criteria for diagnosis

- Negative results on the following tests, if obtained:
- Blood or cerebrospinal fluid cultures blood culture may be positive for *Staphylococcus aureus*
- Negative serologies for Rocky Mountain spotted fever, leptospirosis, or measles

Case classification

- Probable: a case which meets the laboratory criteria and in which four of the five clinical findings described above are present
- Confirmed: a case which meets the laboratory criteria and in which all five of the clinical findings described above are present, including desquamation, unless the patient dies before desquamation occurs

CSTE Position Statement Number: 10-ID-14

Table 3. CDC 1997 Definition for Toxic Shock Syndrome

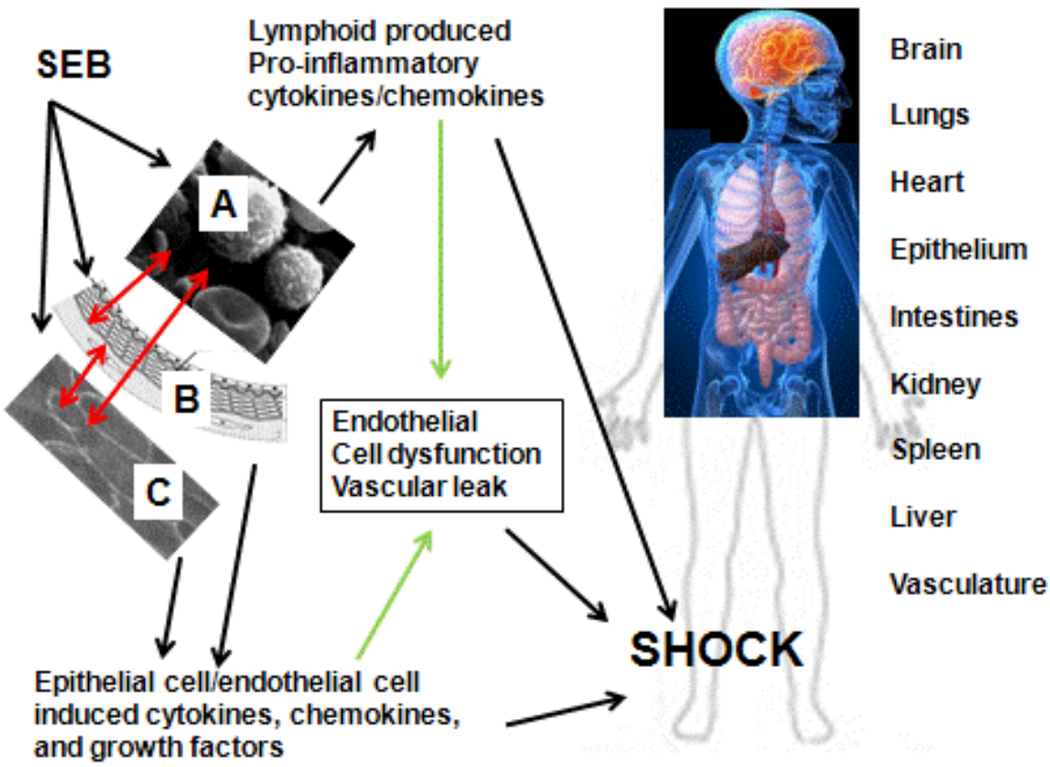

Fig. 3. Systemic SEB (SE) intoxication is a complex disease

SEB interacts with (A) lymphoid cells and induces production of proinflammatory cytokines. SEB stimulates (B) endothelial and (C) epithelial cells to release cytokines (some proinflammatory)/chemokines and growth factors. Red arrows show interactions between cell types are also affected by SEB-induced release of these factors that ultimately leads to endothelial cell dysfunction, vascular leak, and shock resulting from multi-organ failure.

SE binding to lymphoid cells has been extensively characterized and interactions of SEs with these cells are fairly well understood (Achara and Baker, 2004; Broshanan et al. 2011; Larkin et al., 2009). The complexity of the disease suggests that SEs may affect more than lymphoid cells, and recently, an epithelial binding moiety has been identified on the SE molecule (Brosnahan and Schlievert, 20011). SEs bind to epithelial cells and elicit the production of specific cytokines. SEs have also been shown to cross polarized epithelial cells *in vitro* suggesting that SEs gain systemic access to the body (Hale, unpublished data). Rajagpjalan et al. (2007) show acute activation of the systemic immune system and inflammatory response in mice that were vaginally or intranasally exposed to SEB (Rajagopalan et al. 2007; Rajagopalan et al., 2006). Since the toxin was introduced on mucosal surfaces, the only method for systemic activation would be if the toxin crossed the epithelial cell barrier and gained access to the body. Vaginal epithelial cells were also shown to bind TSST-1 and induce TNFα production while treatment of epithelial cells with TSST-1 or SEB induced production of MIP-3α and IL-8 (Brosnahan et al., 2001; Peterson, et al., 2005).

SE-induced shock causes severe damage to the endothelial vasculature and vascular leak contributes significantly to TSS pathology (Krakauer, 1994; Ortega et al. 2010). Elevated levels of vascular endothelial growth factor are observed in serum from patients with sepsis

or septic shock (Karlsson et al. 2008). Because there are common features among hemorrhagic shock, septic shock, and toxic shock syndrome, factors that regulate endothelial homeostasis are probably important in its prevention. Future studies examining the interplay among lymphoid, endothelial and epithelial cells will provide more understanding of the disease and enable a logical approach for therapy.

4.3 Pulmonary complications

One of the most effective and deadly forms of a bioterrorism attack is delivery of the toxin or microorganism by aerosol exposure (Ulrich et al., 1997). Understanding SE-intoxication in humans has been difficult because there is no direct comparison between the pathogenesis of human disease and the disease caused by an intentional aerosol attack. Perhaps the most descriptive and informative reports detailed an accidental laboratory inhalational exposure of fifteen workers (Rusnak et al., 2004). Ten became symptomatic and nine were hospitalized. The onset was rapid (1 1/2 hrs to 24 hr) after exposure with the illness lasting 3-4 days. Commonly observed symptoms were fever, headache, myalgias, pulmonary symptoms, and gastrointestinal symptoms.

A Rhesus macaque animal model was used to characterize SEB intoxication by an aerosol route. In these studies, nonhuman primates (NHP) were exposed to a lethal dose (5 LD50) of aerosolized SEB in a modified Henderson head-only aerosol exposure chamber. NHPs developed gastrointestinal symptoms (anorexia, diarrhea, and emesis) within 24 hr after exposure. The gastrointestinal symptoms appeared to be self-limiting, but 24 hr later, the NHPs developed an abrupt onset of lethargy, dyspnea, and facial pallor. Usually within 4 hr, the animals died or were euthanized when moribund. Postmortem examination revealed lesions in the lungs and signs of pulmonary edema. Both large and small intestines showed petechial hemorrhaging and mucosal erosion, and lymph nodes were swollen. There was definite damage to the endothelium and endothelial cells. The authors of the study concluded that SEB is a potent stimulant in rhesus monkeys and that a similar dose in humans could produce similar symptoms. One thing to consider, however, when extrapolating from NHP to human, is that the NHP were seronegative when tested for the presence of antibodies against SEB; most humans have some degree of past exposure to the toxin and therefore would perhaps have some immunity.

Since these studies on NHP, there is evidence that links SEs exposure to asthma and respiratory problems (Kumar et al., 2010). Inflammatory reactions in the lung are induced by TNF-α and two life-threatening syndromes, vascular leak and respiratory distress develop during toxic shock (Aubert et al., 2000; Herz et al., 1999; Neuman et al., 1997). Other studies show that cytokine-mediated acute respiratory distress syndrome and inflammatory lung disease occur during SE intoxication (DeSouza et al., 2006; Fujisawa et al., 1998; Slifka and Whitton, 2002). Both conditions are critical and may be lethal without therapeutic intervention (Das, 2000; Kasai et al., 1997). Anti-inflammatory drugs reduce TNF-α and other proinflammatory cytokines which alleviates the symptoms and helps the individuals to recover from these life-threatening illnesses.

5. Animal models

A major problem in understanding the disease process of SE intoxication is the lack of appropriate animal models that mimic the human disease. There are several animal models

for studying TSS, but each model has its own limitations, which need to be understood in order to use each model in furthering our understanding of the disease process.

5.1 Mouse

The mouse remains the most common model for TSS studies, although they are not sensitive to the toxin and must be sensitized with either hepatotoxins (e.g., D-galactosamine and actinomycin D) or with endotoxin to achieve an effect (Chen et al., 1994; Nagaki et al., 1994; Blank et al., 1997; Sugiyama et al., 1964). Endotoxin is a natural component of gram-negative bacteria found in the intestines and may actually contribute to shock syndromes. Although tissue damage from SE and lipopolysaccharide (LPS) may vary, acute shock caused by abnormally high levels of TNF-α and other proinflammatory cytokines results in life-threatening situations (Das, 2000; Miekthe et al, 1997; Sifka and Whitton, 2000). Thus, an animal model in which the SEB effects are magnified by sublethal concentrations of LPS provides an *in vivo* system useful for studying various facets of lethal shock. While each mouse model lacks some characteristics of the disease in humans, Krakauer et al. (2010) found that three different mouse models with different susceptibility to SEB could be used to study SEB intoxication.

T-cell deficient mice or mice engineered to have specific cytokine deficiencies show that TNF-α and T cells are both required for SE-induced lethality (Blank, et al. 1997). Transgenic mice expressing human TCR/MHC class II determinants solve some problems associated with mouse lymphoid cells binding SE and SAG-sensitive mice show a biphasic release of cytokines with early TNF-a release mediating lethal shock (Faulkner et al., 2005; Rajagopalan et al., 2002). These investigations point also to the spleen as a major source of TNF-a production during an acute (early) cytokine response. The studies support the idea that TSS is not simply due to cytokines released by T cells, but entails a series of events affecting major organs throughout the body. Recently, a humanized mouse in which T-cell immune deficient mice, SCID, were transfused with human hematopoietic fetal liver CD34+ cells that had previously been implanted with human fetal thymic and liver tissues developed long-term human innate and adaptive immune responses. When TSST-1 was injected into these mice, the mice responded immunologically in a manner similar to humans (Melkus et al., 2006) suggesting that this mouse model may overcome many of the problems associated with mouse models for SE intoxication.

5.2 Rat

Rats have been an excellent model to study TSS effects on the nervous system. Wang et al, (2004) showed activation of neuronal developmental genes after rats were given intraperitoneal injections of SEB. Activation appeared to occur through the tenth cranial nerve, the vagus, because severing this nerve prevented neuronal activation. These studies support the idea that brain-immune system communications play a role in TSS. Some sequalae of TSS relate to memory loss and confusion which would indicate involvement of the nervous system (Kusnocov and Goldfaith, 2005).

5.3 Minature swine

Because a major drawback for murine and rodent models in TSS is the lack of clinical symptoms that occur in humans, a porcine model has been developed in which 18-day-old

piglets are given a lethal dose of SEB intravenously (van Gessel et al., 2004). Intoxicated piglets develop vomiting, diarrhea, febrile temperature spikes, anorexia, and hypotension similar to the clinical course of disease observed in humans and may offer an *in vivo* model with more of the symptoms observed in human TSS.

5.4 Rabbit

McCormick et al. (2003) noted that rabbits, when given SAG by a continuous perfusion, developed pyrogenic symptoms similar to humans. Investigations showed that the lethal pathology was similar to that observed in human TSS, but with newer animal models and a greater understanding of mechanisms involved in TSS, rabbits are not a major animal model for TSS.

5.5 Shrew

Most of the small animal models used to study the effects of SE-intoxication do not display an emetic response (King, 1990). Hu et al. (2003) developed an emetic model with which to study SE-induced emesis. They reported that several SE serotypes, known to induce emesis in NHP, induced emesis in the house musk shrew (*Suncus murinus*). Concentrations required to induce an emetic response were approximately 0.4 µg per animal, but the dose varied with the SE serotype. Variations in SE toxicity among the serotypes were similar to those observed in NPHs and humans, making the shrew an excellent small animal model to study emesis induced by SEs.

5.6 Nonhuman primate

NHP exhibit a similar disease progression as that observed in humans and are susceptible when given SEB orally (Boles et al., 2003; Mattix et al., 1995; Ulrich et al., 1997). Because there are limitations in the number of NHP available for studies and because of the expense involved in NHP studies, they are usually reserved for preclinical investigations. While TSS manifestations in NHPs resemble those observed in humans, there are differences between the immune systems, which may become more evident as more is learned about the disease process.

6. Therapy and prophylaxis

Most therapeutic and prophylatic measures are concerned with TSS or systemic SE intoxication because food poisoning is usually self-limiting. Although identification of unusual food poisoning incidents should be monitored as a possible biothreat action, the disease itself should not be life-threatening, and recovery occurs without serious side effects. Because therapy for food poisoning is not a serious concern, this section will address the measures to treat disease pathogenesis resulting from systemic SE intoxication.

6.1 Current therapeutic measures

At the current time, intravenous human gamma globulins (IVIG) is the primary therapeutic to treat TSS. Because there are no specific drugs available for treatment, a primary goal of any therapeutic intervention is to maintain important body functions and physiological

homeostasis. Recommendations for treating TSS include first the removal of any foreign materials that might be contaminated with *S aureus* (i.e., tampons or nasal packings) and draining sites of infection to prevent further bacterial growth. Treatment with anti-microbials is also recommended if sepsis is involved. In severe cases, other therapeutic interventions that may minimize the risk of tissue damage and organ failure include fluids to prevent dehydration, dialysis if severe kidney problems occur, drugs to control blood pressure and cardiovascular function, anti-inflammatory agents, and possibly insulin, if needed. Supportive care should be aggressive and monitored carefully. Length of hospitalization may vary between 5 and 11 days (DeVries et al., 2011).

6.2 Antibody therapy for SE intoxication

As shown by studies in NHP, animals could be protected against SE intoxication using passive immunization with anti-SEB antibodies. The antibodies provided protection if given up to 4 hr after the NHPs had received 5 LD50sof aerosolized SEB (LeClaire et al., 2002). These studies showed that the antibodies were able to neutralize the toxin *in vivo* and provide protection after intoxication had occurred. Unfortunately, the antibody was a monoclonal antibody (Mab) created in a chicken, and the antibody itself may be antigenic and cause serum sickness. There are several anti-SEB Mab preparations that will not cause adverse reactions in humans. Because most Mabs are not developed using human cells, Mab development for human use will require "humanization of the Mab in order to eliminate the molecule's antigenicity (Goldsmith and Signore, 2010). Investigations using immune lymphocytes to prepare Mabs may provide therapeutics without the expense of humanization. Additionally, developing small-domain antibody fragments such as camilid antibodies may also provide therapeutic agents that, because of their size, may not be antigenic in humans (Graef et al., 2011). Further studies are needed to identify antibody reagents that will be successful therapeutics, and whether the antibody reagents will need to be combined with pharmacologic agents to enhance their efficacy.

6.3 Possible pharmacologic agents to treat SE intoxication

Over the past 20 years, there have been numerous investigations showing efficacy of various pharmacologic agents that prevent or delay lethality when animals are treated after a SE challenge, and as yet none has advanced to human clinical trials (Krakauer, 2010). Human activated protein C (hAPC) was approved for patients in severe septic shock, and had been used to treat TSS (DeVries et al. 2011). On October 25, 2011, Eli Lilly withdrew *Xigris (hAPC) because more in depth clinical trials indicated that drug did no better than a placebo in reducing mortality (*http://www.medscape.com/viewarticle/752169*).* The complexity of TSS tends to discount development of a single drug capable of treating the disease . Because the disease affects the endothelial cell vasculature of multiple organ systems, including drugs that treat endothelial cell damage should also help to establish a therapeutic regimen for TSS.

6.4 Vaccines

To protect soldiers against SEB exposure, the U. S. Army developed a formalin-treated SEB toxoid that had some degree of success in protecting animals against a SEB challenge (Tseng et al., 1995). Due to the fact that formalin treatment did not always inactivate every toxin molecule, there was a move to develop vaccines without the need for formalin

inactivation. A vaccine developed by site-specific mutagenesis provided safer and more effective vaccines. The most effective vaccine (SEBvax) was designed with mutations in the MHC class II binding region so that the vaccine no longer was capable of cross-linking T cells to APCs (Ulrich et al., 1998). The military is no longer funding development of SEBvax. The vaccine is now being used to develop a trivalent subunit vaccine that includes mutated Tst-1 and SEA proteins as well. The combination vaccine should provide protection against SEB but also against SEs more commonly associated with TSS (http://www.regionalinnovation.org/success.cfm?story=32).

7. Detection of SEs and SEB

Since September 11, 2001, The U.S. and its allies have been concerned with detection of those agents that could be dispersed by aerosol (Kman and Bachman, 2011). There are several methods used for monitoring agents of bioterrorism and surveillance occurs through an umbrella of monitoring systems. A major component of surveillance, syndromic surveillance, results from the monitoring of clinical manifestations of certain illnesses to determine if there is a higher than normal number of cases. This is usually followed by laboratory surveillance in which certain markers and laboratory data indicate the presence of a bioterrorism agent. Another type of surveillance is environmental during which the environment is continually sampled for the presence of biological agents. In situations like SEB which is not generally monitored environmentally via the BioWatch Program, syndromic and laboratory surveillance becomes extremely important for monitoring bioterrorism attacks.

SEs are stable proteins and therefore identification of the proteins using anti-SEB reagents should be possible in both the field and medical facilities. Available immunoassays are capable of identifying the protein in picogram amounts and can be used to monitor samples taken from the environment (Kahn et al. 2003; Sapsford et al., 2005). Because most humans have been exposed to SEs and have developed antibodies against them, the presence of anti-SE antibodies is of little diagnostic value, but the detection of the toxin in body fluids or from nasal swabs (after an aerosol exposure), should provide a positive confirmation (Ulrich et al., 1997).

In cases of infection with *S. aureus*, polymerase chain reaction (PCR) assays can determine the presence of the SE gene (Chiang et al., 2008; Rajkovic et al., 2006). Particularly in the case of a toxin that is known to be present in staphylococcal infections, surveillance and monitoring at the clinical level is imperative for differentiating between random outbreaks of the disease and a bioterrorist attack.

8. Category B biothreat agent

Although redefined after September 11, 2001, rogue nations have used bioterrorism for centuries as a method to harm their opponents (Bellamy and Freedman, 2001; Phillips, 2005). As described by CDC, bioterrorism agents are separated into three categories for preparedness purposes depending upon their ease of dissemination, and the ability to cause excessive morbidity and mortality (Rotz et al, 2002). Category A includes agents such as Variola major (smallpox) and *Yersinia pestis* (plague) that have been used as a weapon of mass destruction (WMD) (Henderson, 1999). As previously mentioned, Category B agents

are easy to disseminate and produce moderate morbidity and low mortality. Category B agents do not meet criteria for use as a WMD, but dispersal of a Category B agent could result in regional disruptions and hysteria.

From all accounts, SEB meets the criteria for a Category B agent in that it is stable, easy to disseminate, and induces severe emesis and toxic shock. An aerosol of SEB in a crowded area could lead to an incapacitating disease in several hundred individuals. Although mortality would be low, the illness would create a serious public health impact by disrupting normal work days and cause havoc by increasing individual use of emergency rooms (Ulrich et al., 1997).

Many bioterrorism agents such as SEB are found in nature, are easy to isolate and produce in mass quantities and are usually stable in adverse environmental conditions (Ahanotu, et al., 2006). Because the agent is a common inhabitant in the environment, monitoring the agent becomes more difficult. The fact that there are accidental cases of food poisoning and occasional cases of TSS annually also complicates identifying bioterrorism incidents using SEB. In the final analysis, although SEB may not be the most favored bioterrorism agent, there is always a possibility that it will be used in an attack and, therefore, mechanisms should be in place for decontamination and treatment.

9. Summary

SEs are produced primarily by *S.aureus* which is a common inhabitant in the environment worldwide. SEs are a major cause of food poisoning and toxic shock syndrome. In the 1960s, SEB was weaponized as an incapacitating agent, and now is listed as a Category B bioterrorism agent. When inhaled, the toxin causes severe respiratory damage and endothelial dysfunction, often resulting in acute respiratory distress and severe lung damage. As yet, there is no FDA-approved vaccine or therapeutic agents to prevent or treat SEB-intoxication and with its ease of dissemination, SEB remains a serious bioterrorism agent.

10. Acknowledgements

The work was supported by funds from the Defense Threat Reduction Agency (project CBM.THRTOX.03.10.RD.020). The opinions, interpretations, conclusions, and recommendations expressed in this publication are those of the author and are not necessarily endorsed by the US Army.

11. References

Abrahmsen, L., Dohlsten, M., Segren, S., Bjork, P., Johsson, E., and Kalland, T. (1995) Characterization of two distinct MHC class II binding sites in the superantigen staphylococcal enterotoxin A. EMBO J. 14: 2978-2986.

Acharya K. R., Baker M. D., (2004) Superantigen: structure-function relationships. Int. J. Med. Microbiol. 293: 529-37.

Alber, G, Hammer, K., and Fleischer, B. (1990) Relationship between enterotoxic and T lymphocyte-stimulating activity of staphylococcal enterotoxins. *J. Immunol*. 144:4501-4506.

Alouf, J. E. and Muller-Alouf, H. (2003) Staphylococcal and streptococcal superantigens: molecular, biological, and clinical aspects. *Int. J. Med. Microbiol*. 292:429-440.

Ahanotu, E., Alvelo-Ceron, D., Ravita, T., Gaunt, E. (2006) Staphylococcal enterotoxin B as a biological weapon: recognition, management, and surveillance of staphylococcal enterotoxin. Appl. Biosafety 11: 120-176.

Aubert, V., Schneeberger D., Sauty A., Winter J., Sperisen P., Aubert J., and Spertini F. (2000) Induction of tumor necrosis factor alpha and interleukin-8 gene expression in bronchial epithelial cells by toxic shock syndrome toxin 1. Infect. Immun. 68:120-124.

Baker, M. D. and Acharya, K. R. (2004) Superantigens: structure-function relationships. *Int. J. Med. Microbiol*. 293: 529-537.

Bellamy, R. J. and Freedman, A. R. (2001) Bioterrorism. Q J Medical J. 94: 227-234.

Bergdoll, M. S., Surgalla, M. J., and Dack, G. M. (1959) Staphylococcal enterotoxin I. purification. Arch. Biochem. Biophys. 85: 62-69.

Bergdoll, M. S., Borja, C. R., and Avena, R. M. (1965) Identification of a new enterotoxin as enterotoxin C. J. Bacteriol. 90: 1481-1485.

Bergdoll, M.S., Huang, I. Y., and Schantz, E. J. (1974) Chemistry of the staphylococcal enterotoxins. J. Agric. Food Chem. 22: 9-13.

Bergdoll, M. S. (1983) Enterotoxins. In: *Staphylococci and Staphylococcal Infections* (Easman, C.S.F. and Adlam, C., eds.). Academic Press, London, UK, pp. 559-598.

Bergdoll, M. S. (1989) *Staphylococcus aureus*. In: *Foodborne Bacterial Pathogens* (Doyle, M.P., ed.). Marcel Dekker, Inc., New York, NY, USA, pp. 463-523.

Blank, C., Luz, A., Bendigs, S., Erdmann, A., Wagner, H., and Heeg, K. (1997) Superantigen and endotoxin synergize in the induction of lethal shock. Eur. J. Immunol. 27: 825-833.

Boles, J. W., Pitt, M. L., LeClaire, R. D., Gibbs, P. H., Torres, E., Dyas, B., Ulrich, R. G., Bavari, S. (2003) Generation of protective immunity by inactivated recombinant staphylococcal enterotoxin B vaccine in nonhuman primates and identification of correlates of immunity. Clin. Immunol. 108: 51-59.

Brosnahan, A. J. and Schievert, P. M. (2011) Gram-positive bacterial superantigen outside-in signaling causes toxic shock syndrome. FEBS J. 278:4649-4667.

Brosnahan, A. J., Mantz, M. J., Squier, C. A., Peterson, M. L. and Schlievert, P. M. (2009) Cytolysins augment superantigen penetration of stratified mucosa. J. Immunol. 182: 2364-2373.

Canaan, A., Marcus, H., Burakova, T., David, M., Dekel, B., Segal, H., and Reisner, Y. (1999) T cell control of staphylococcal enterotoxin B (SEB) lethal sensitivity in mice: CD4+CD459bright)/CD4+Cd45RB(dim) balance defines susceptibility to SEB cytotoxicity. Eur. J. Immunol. 29: 1375-1382.

Casman, E. P. (1960) Further serological studies of staphylococcal enterotoxin. J. Bacteriol. 79: 849-856.

Casman, E. P., Bennett, R. W., Dorsey, A. E., and Issa, J. A. (1967) Identification of a fourth staphylococcal enterotoxin, enterotoxin D. J. Bacteriol. 94: 1875-1882.

Chiang, Y. C., Liao, W. W., Fan, C. M., Pai, W. y. Chiou, C. S., and Tsen, H. Y. (2008) PCR detection of staphylococcalenterotoxins (SEs) N, O, P, Q, R, U and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. Int. J. Food Microbiol. 15: 66-73.

Chen, J. Y., Qiao, Y., Komisar, J. L., Baze, W. B., Hsu, I. C., and Tseng, J. (1994) Increased susceptibility to staphylococcal enterotoxin B in mice primed with actinomycin D. Infect. Immun. 62: 4626-4631.

Chesney, P. J., Davis, J. P., Purdy, W. K., Wand, P. J., and Chesney, R. W. (1981) Clinical manifestations of toxic shock syndrome. JAMA 246: 741-748.

Choi, Y. W., Kotzin, B., Herron,L., Callahan, J., Marrack, P., and Kappler, J. (1989) Interaction of Staphylococcus aureus toxin "superantigens" with human T cells. Proc Natl Acad Sci U S A. 86: 8941–8945.

Croddy, E. C., Hart, C., and Perez-Armendariz J., *Chemical and Biological Warfare*, (Google Books), Springer, 2002,pp. 30-31, (ISBN 0387950761

Dack, G. M., Cary, W. E., Wollpert, O., and Wiggins, H. J. (1930) An outbreak of food poisoning proved to be due to a yellow haemolytic Staphylococcus. J. Prev. Med. 4: 167-175.

da Cunha, M., Calsolari, R. A., and Junior, J. P. (2007) Detection of enterotoxin and toxic shock syndrome toxin 1genes in Staphylococcus, with emphasis on coagulase-negative staphylococci. Microbiol. Immunol. 51: 381-390.

Das, U. N. (2000) Critical advances in septicemia and septic shock. Crit. Care 4:290–296.

Davis, J. P. Chesney, P.J., Wand, P.J., and LaVenture M. (1980) Toxic-shock syndrome: epidemiologic features, recurrence, risk factors, and prevention. N. Engl. J. Med. 303, 1429-1435.

Desouza, I. A., Franco-Penteado, C. F., Camargo, E. A., Lima, C. S., Teixeira, S., Muscara, M. N., De Nucci, G., and Antunes, E. (2006) Acute pulmonary inflammation induced by exposure of the airways to staphylococcal enterotoxin type B in rats. Toxicol. Appl. Pharmacol. 15: 107-113.

DeVries, A. S., Lesher, L., Schlievert, P. M., Rogers, T., Villaume, L. G., Danilla, R., and Lynfield, R. (2011) Staphylococcal toxic syndrome 2000-2006: epidemiology, clinical features, and molecular characterization.

Dinges, M. M., Orwin, P, M., and P. M. Schlievert, P. M. (2000) Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev. 13: 16-34.

Do Carmo, L. S., Cummings, C., Linardi, V. R., Dias, R. S., De Souza, J. M., Sena, M. J., Dos Santos, D. A., Shupp, J. U., Poreira, R. K., Jett, M. (2004) A case study of a massive staphylococcal food poisoning incident. Foodborne Pathog. 1: 241-246.

Fast, D. J., Schlievert, P. M., and Nelson, R. D. (1989) Toxic shock syndrome-associated staphylococcal and Streptococcal pyrogenic toxins are potent inducers of tumor necrosis factor production. Infect. Immun. 57: 291–294.

Faulkner, L., Cooper, A., Fantino, C., Altmann, D.M., and Sriskandan, S. (2005) The mechanism of superantigen-mediated toxic shock: not a simple Th1 Cytokine storm. J. Immunol. *175*: 6870-6877.

Fournier, B. (2008) Global regulation of *Staphylococcal aureus* virulence genes. In: Lindsay, J. S. (ed). Staphylococcus molecular genetics. Caister Academic Press, Norfolk, UK, pp. 131-183.

Franz, D. R., Parrott, C. D., and Takafuji, E. T. (1997) The U.S. biological warfare and biological defense programs, p. 425-436. *In* Textbook of military medicine. Part I. Warfare, weaponry and the casualty, vol. 3. U.S. Government. Printing Office, Washington, D.C.

Fraser, J. D., Urban, R. G., Strominger, J. L., and Robinson, H. (1992) Zinc regulates the function of twosuperantigens. Proc. Natl. Acad. Sci. 89: 5507-5511.

Fujisawa, N., Hayashi, S., Kurdowska, A., Noble, J. M., Naitoh, K., and Miller, E. J. (1998) Staphylococcal enterotoxinA injury of human lung endothelial cells and IL-8 accumulation are mediated by TNF-α. J. Immunol. 161:5627–5632

Goldsmith, S. J. and Signore, A. (2010) An overview of the diagnostic and therapeutic use of monoclonal antibodies in medicine. Q. J. Nucl. Mol. Imaging. 54: 574-581.

Graef, R. R., Anderson, G. P., Doyle, K. A., Zabetakis, D., Sutton, F. N., Liu, J. L., Serrano-González, J., Goldman, E. R., and Cooper, L. A. (2011) Isolation of a highly thermal stable lama single domain antibody specific for *Staphylococcus aureus* enterotoxin B. BMC Biotechnol. Published online 2011 September 21. doi: 10.1186/1472-6750-11-86.

Greenfield, R. A., Brown, B. R., Hutchins, J. B., Iandolo, J. J., Jackson, R., Slater, L. N., and Bronze, M. S. (2002) Microbiological, biological, and chemical weapons of warfare and terrorism. Am. J. Med. Sci. 323: 326-340.

Haffner, A. C., Zepter, K., and Elmets, C. A. (1996) Major histocompatability complex class I serves as a ligand for presentation of the superantigen enterotoxin B to T cells. Proc. Natl. Acad. Sci. 93: 3037-3042.

Hamad, A. R. A., Marrack, P., and Kappler, J. W. (1997) Transcytosis of staphylococcal enterotoxins. J. Exp. Med. 185: 1447-1454.

Henderson, D. A. (1999) The looming threat of bioterrorism. Science 283: 1279-1282.

Herz, U., Ruckert, R., Wollenhaupt, K., Tschernig, T., Neuhaus-Steinmetz, U., Pabst, R., and Renz, H. (1999) Airwayexposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness--a model for non-allergic asthma. Eur.J. Immunol. 29: 1021–1031.

Horshburg, M. J. (2008) The response of *S. aureus* to environmental stimuli. In: Lindsay, J. S. (ed). Staphylococcus molecular genetics. Caister Academic Press, Norfolk, UK, pp. 185-206.

Hursh S, McNally, R., Fanzone, J. Jr, and Mershon, M. (1995) Staphylococcal Enterotoxin B Battlefield ChallengeModeling with Medical and Non-Medical Countermeasures. Joppa, Md: Science Applications International Corp; Technical Report MB DRP-95-2.

Hu, D. L., Zhu, G., Mori, F., Omoe, M., Wakabayashi, K., Kaneko, S., Shinagawa, K., and Nakane, A. (2007)Staphylococcal enterotoxin induces emisis through increasing serotonin release in intestine and it is downregulated by cannabinoid receptor 1. Cell. Microbiol. 9: 2267-2277.

Hu, D. L., Omoe, K., Shimoda, Y., Nakane, A., and Shinagawa, K. (2003) Induction of emetic response tostaphylococcal enterotoxin in the House Musk Shrew (Suncus murinus). Infect. Immun. 71: 567-570.

Jardetzky, T. S., Brown, J. H., Gorga, J. C., Stern, L. J., Urban, R. G., Chi, Y. I., Stauffacher, C., Strominger, and J. L., Wiley, D. C. (1994) Three-dimensional structure of a human class II histocompatibility molecule complexed with superantigen. Nature 368: 711-718.

Jarraud, S., Peyrat, M. A., Lim, A., Tristan, A., Bes, M., Mougel, C., Etienne, J., Vandenesch, F., Bonneville, M., and in Staphylococcus aureus. J. Immunol. 166: 669-677.

Kasai, T., Inada K., Takakuwa T., Yamada Y., Inoue Y., Shimamura T., Taniguchi S., Sato S., Wakabayashi G., and Endo S. (1997) Anti-inflammatory cytokine levels in patients with septic shock. Res. Commun. Mol. Pathol. Pharmacol. 98:34–42.

Karlson, S, Pettila, V., Tenhunen, J., Lund, V., Hovilehto, S., and Ruokonen, E. (2008) Vascular endothelial cell growth factor in severe sepsis and septic shock. Anesth. Analg. 106: 1820-1826.

Kerouanton, A., Hennekinne, J. A., Letertre, C., Petit, L., Chesneau, O., Brisabois, A., and De Buyser, M. L. (2007)Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. Int. J. Food Microbiol. 115: 369-375.

Khan, A. S., Cao, C, J., Thompson, R. G., and Valdes, J. J. (2003) A simple and rapid fluorescence-based immunoassay for the detection of staphylococcal enterotoxin B. Mol. Cell. Probes 17: 125–126.

King, G. L. (1990) Animal models for studying vomiting. Can. J. Physiol. Pharmacol. 68: 260-268.

Kissner (a), T. L., Ruthel, G., Alam, S., Ulrich, R. G., Fernandez, S., and Saikh, K. U. (2011) Activation of MyD88 signaling upon staphylococcal enterotoxin binding to MHC class II molecules. Plos One 20: e15985.

Kissner (b), T. L., Ruthel, G., Cisney, E. D., Ulrich, R. G., Fernandez, S., and Saikh, K. U. (2011) MyD88-dependent pro-inflammatory cytokine response contributes to lethal toxicity of staphylococcal enterotoxin B in mice. Innate Immun. 17: 451-462.

Kman, N. E. and Bachmann, D. J. (2011) Biosurveillance: a review and update. Adv. Preventive Med. www.hindawi.com/journals/apm/aip/301408/ -

Krakauer, T. (2010) Therapeutic down-modulators of staphylococcal superantigen-induced inflammation and toxic shock. Toxins: 2: 1963-1983.

Krauker, T., Buckley, M., and Fisher, D. (2010) Murine models of staphylococcal enterotoxin B-induced toxic shockMil. Med. 175: 917-922.

Krakauer, T. (1994) Costimulatory receptors for the superantigen staphylococcal enterotoxin B on human vascular endothelial cells and T cells. J. Leukocyte Biol. 56: 458-463.

Kumar, S., Menoret, A., Ngoi, S., and Vella, A. T. (2010) The systemic and pulmonary immune response to staphylococcal enterotoxins. Toxins 2: 1898-1912.

Kusnecov, A. W. and Goldfarb, Y. (2005) Neural and behavorial responses to systemic immunologic stimuli: a consideration of bacterial T cell superantigens. Curr. Pharm. Des. 11: 1039-1046.

Larkin, E. A., Carman, R. J., Krakauer, T., and Stiles, B. G. (2009) *Staphylococcus aureus*: The toxic presence of a pathogen extraordinaire. Cur. Medicinal Chem. 16: 4003-4019.

LeClaire, R. D., Hunt, R. E., and Bavari, S. (2002) Protection against bacterial superantigen staphylococcal enterotoxin B by passive vaccination. Infect. Immun. 70: 2278-2285.

Le Loir, Y., Baron, F., and Gautier, M. (2003) *Staphylococcal aureus* and food poisoning. Genet. Mol. Res. 2: 630-76.

Li, Y., Li, H., Dimasi, N., McCormick, J. K. and Martin, R. (2001) Crystal structure of a superantigen bound to the high-affinity zinc-dependent site on MHC class II. Immunity 14: 93-104.

Lindsay, J. A. (2011) Genomics of *Staphylococcus*. pp. 243-267 in Genomics of Foodborne Pathogens, Weidman, M. and Zhang, W. Springer Science+Business Media, Springer, New York, New York.

McCormick, J. K., Yarwood, J. M. and Schlievert, P. M. (2001) Toxic shock syndrome and bacterial superantigens: an update. Annu. Rev. Microbiol. 55: 77-104.

McCormick, J. K. Bohach, G. A., and Schlievert, P. M. (2003) Pyrogenic, lethal and emetic properties of superantigens in rabbits and primates. Meth. Mol. Biol. 214: 245-253.

Magnotti, L. J., Upperman, J. S., Xu, D. Z. Lu, Q., and Deitch, E. (1998) Gut-derived mesenteric lymph but not portal blood increases endothelial cell permeability and promotes lung injury after hemorrhagic shock. Ann. Surg. 228: 518-527.

Marrack, P., Blackman, M., Kushnir, E., and Kappler, J. (1990) The toxicity of staphylococcal enterotoxin B in mice is mediated by T cells. J. Exp. Med. 171: 455-464.

Marrack, P. and Kappler, J. (1990) The staphylococcal enterotoxins and their relatives. Science. 248: 705-709.

Mattix, M. E., Hunt, R. E., Wilhelmsen, C. L., Johnson, A. J., and Baze, W. B. (1995) Aerosolized staphylococcal enterotoxin B-induced pulmonary lesions in rhesus monkeys (*Macaca mulatta*). Toxicol. Pathol. 23: 262-268.

Melkus, M. W., Estes, J. D., Padgett, T. A., Gatlin, J., Denton, P. W., Wege, A. K., Hasse, A. T. and Garcia, J. V. (2006) Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat. Med. 12: 1316-1322.

Miethke, T., Wahl, C., Heeg, K., Echtenacher, B., Krammer, P. H., and Wagner, H. (1992) T cell-mediated lethal shock triggered in mice by the superantigen superantigen staphylococcal enterotoxin B: critical role of tumor necrosis factor. J. Exp. Med. 175: 91–98

Nagaki, M., Muto, Y., Ohnishi, H., Yasuda, S., Sano, K., Naito, T., Maeda, T., Yamada, T., and Moriwaki, H. (1994) Hepatic injury and lethal shock in galactosamine-sensitized mice induced by the superantigen staphylococcal enterotoxin B. Gasteroenterology 106: 450-458.

Nagashima, T., Aranamai, T., Iclozan, C., and Onoe, K. (2004) Analysis of T Cell responses to a superantigen, staphylococcal enterotoxin-B. J. Clin. Exp. Hematopathol. 44: 25-32.

Neumann, B., Engelhardt, B., Wagner, H., and Holzmann, B. (1997) Induction of acute inflammatory lung injury by Staphylococcal enterotoxin B. J. Immunol. 158: 1861-1871.

Nout MJ., Notermans S., and Rombouts, FM. (1988) Effect of environmental conditions during soya-beanfermentation on the growth of *Staphylococcus aureus* and production and thermal stability of enterotoxins A and B. Int J. Food Microbiol. 31: 299-309.

Ono, H. K., Omoe, K., Imanishi, K., Iwakabe, Y., Hu, D., Kato, H., Saito, Naoyuki, Nakane, A., AUchiyama, T., andShinagawa, K. (2008) Identification and characterization of two novel staphylococcal enterotoxins, Types S and T. Infect. Immun. 76: 4999-5005.

Ortega, E., Abriouel, H., Lucas, R., and Galvez, A. (2010) Multiple roles of *Staphylococcus aureus* entertoxins: pathogenicity, superantigenic activity, and correlation to antibiotic resistance. Toxins 2: 2117-2131.

Otero, A., Garcia, M. L., Garcia, M. C., Moreno, B., and Bergdoll, M. S. (1990) Production of staphylococcal enterotoxins C1 and C2 and thermonuclease throught the growth cycle. Appl. Envion. Microbiol. 56: 555-559.

Papageorgiou, A. C., Acharya, K. R. (2000) Microbial superantigens: from structure to function. Trends Microbiol. 8: 369-75.

Papageorgiou, A. C., Tranter, H. S., and Acharya, K. R. (1998) crystal structure of microbial superantigen enterotoxin B at 1.5 A resolution: implications for superantigen recognition by MHC class II molecules and T-cell receptors. J. Mol. Biol. 277: 61-79.

Peterson, M. L., Ault, K., Kremer, M. J., Klingelhutz, A. J., Davis, C. C., Squier, C. A., and Schlievert P. M. (2005) The innate immune system is activated by stimulation of

vaginal epithelial cells with *Staphylococcus aureus* and toxic shock syndrome toxin 1. Infect. Immun. 73: 2164-2174.

Phillips, M. D. (2005) Bioterrorism: a brief history. Focus on Bioterrorism. www.DCMSonline.org.

Pinchuk, I. V., Beswick, E. J., and Reyes, V. E. (2010) Stapylococcal enterotoxins. Toxins: 2: 2177-2197.

Ples, D. D., Ruthel, G., Reinke, E. K., Ulrich, R. G., and Bavari, S. (2005) Persistance of zinc-binding bacterial superantigens at the surface of antigen-presenting cells contributes to the extreme potency of these superantigens as T-cell activators. Infect. Immun. 73: 5358-5366.

Rajagopalan, G., Smart, M. K., Krco, C. J., and David, C. S. (2002) Expression and function of transgenic HLA-DQ molecules and lymphocyte development in mice lacking invariant chain. J. Immunol. 169: 1774-1783.

Rajagopalan, G., Smart, M. K., Murali, N., Patel, R., and David, C. S. (2007) Acute systemic immune activation following vaginal exposure to staphylococcal enterotoxin B— implications for menstrual shock. J. Reprod. Immunol. 73: 51-59.

Rajagopalan, G., Sen, M. M., Singh, M., Murali, N., Nath, K. A., Iijima, K., Kita, H., Leontovich, A. A., Gopinathan,U., Patel, R., and David, C. S. (2006) Internasal exposure to staphylococcal enterotoxin b elicits an acute systemic inflammatory response. Shock 25: 647-656.

Sapsfors, K. E., Taitt, C. R., Loo, N., and Frances, S. (2005) Biosensor detection of botulinum toxoid A and staphylococcal enterotoxin in food. Appl. Environ. Microbiol. 71: 5590-5592.

Schad, E. M., Papageorgiou, A., C., Svensson, L. A., and Acharya, K. R. (1997) A structrural and functional comparison of staphylococcal enterotoxin A and C2 reveals remarkable similarity and dissimilarity J. Mol. Biol. 269: 270-280.

Soejima, T., Nagau, E., Yano, Y., Yamagata, H., Dagi, H., Sinagawa, K. (2007) Risk evaluation for staphylococcal food poisoning in processed milk produced with skim milk powder. Int. J. Food Microbiol. 115: 29-34.

Slifka, M. K., and J. L. Whitton. (2000) Clinical implication of dysregulated cytokine production. J. Mol. Med. 78:74–80.

Stevens, D. L. (1997) Streptococcal toxic shock syndrome. In: Leung DYM, Huber BT, Schlievert PM, eds. Superantigens: Molecular Biology, Immunology, and Relevance to Human Disease. New York, NY: Marcel Dekker, Inc; pp. 481–501.

Suda, T., Sato, A., Sugiura, W., and Chida, K. (1995) Induction of MHC class II antigens on rat bronchial epithelial cells by interferon-gamma and its effect on antigen presentation. Lung. 173:127-137.

Sundstrom, M., Abrahmsen, I., Antonsson, P., Mehindate, K., Morad, W., Mchindate, K., and Dohlsten, M. (1996)The crystal structure of staphylococcal enterotoxin type D reveals Zn^{2+}-mediated homodimerization. EMBO J. 15: 6832-6840.

Sugiyama, H., McKissic, E. M., Bergdoll, M. S., and Heller, B. (1964) Enhancement of bacterial endotoxin lethality by staphylococcal enterotoxin. J. Infect. Dis. 114: 111–118.

Rajkovic, A., El-Mousalij, B., Uynendaele, M., Brolet, P., Zorzi, W., Heinen, E., Foubert, E., and Debevete, J.(2006) Immunoquantitative real-time PCR for detection and quantification of staphylococcal enterotoxin B in foods. Appl. Environ. Microbiol. 72: 6593-6599.

Rotz, L. D., Khan, A. S., Lillibridge, S. R., Ostroff, S. M., and Hughes, J. M. (2002) Public health assessment of potential biological terrorism agents. *Emerg. Infectious Dis.* 8: 225-30.

Rusnak, J. M., Kortepeter, M., Ulrich, R., Poli, M., and Boudreau, E. (2004) Laboratory exposures to staphylococcal nterotoxin B. Emerg. Infect Dis.10: 1544– 1549.

Thomas, D. V., Jarraud, S., Lemercier, B., Cozon, G., Echasserieau, K., Etienne, J., Gougeon, M. I., Lina, G., and Vandensch, F. (2006) Staphylococcal enterotoxin-like toxins U2 and V, two new staphylococcal superantigens arising from recombination within the enterotoxin gene cluster. Infect. Immun. 74: 4724-4734.

Todd, J., Fishaut, M., Kapral, F., and Welch, T. (1978) Toxic-shock syndrome associated with phage-group 1 staphylococci. Lancet 2: 1116-1118.

Tseng, J., Komisar, J. L., Trout, R. N., Hunt, R. E., Chen, J. Y., Johnson, A. J., Pitt, L., and Ruble, D. L. (1995) Humoral immunity to aerosolized staphylococcal enterotoxin B (SEB), a superantigen, in monkeys vaccinated with SEB toxoid-containing microspheres. Infect. Immun. 63: 2880-2885.

Uchiyama, T., Imanishi, K., Miyoshi-Akiyama, T, and Kato, H. (2006) In: The Comprehensive Sourcebook of Bacterial Protein Toxins, J. E. Alouf and M. R. Popoff, Eds: Academic Press, Paris, pp. 830-843.

Ulrich, R. G., Olson, M. A., and Bavari, S. (1998) Development of engineered vaccines effective against structurally related bacterial superantigens. *Vaccine* 16: 1857-1864.

Ulrich, R. G., Sidell, S., Taylor, T. J., Wilhelmsen, C. L., and Franz, D. R. (1997) Staphylococcal enterotoxin B and related pyrogenic toxins, p. 621-631. *In* Textbook of military medicine. Part I. Warfare, weaponry and the casualty, vol. 3. U.S. Government. Printing Office, Washington, D.C.

van Gessel Y. A., Mani, S., Bi, .S, Hammamieh, R,, Shupp, J.W., Das, R., Coleman, G.D., and Jett, M. (2004) Functional piglet model for the clinical syndrome and postmortem findings induced by staphylococcal enterotoxin B. *Exp. Biol. Med*. 229: 1061-1071.

Wang, B. R., Zhang, X. J., Duan, X. L., Guo, X., and Ju, G. (2004) Fos Expression in the rat brain after intraperitoneal injection of Staphylococcus enterotoxin B and the effect of vagotomy. Neurochem. Res. 29: 1667-1674.

Wen, R., Cole, G. A., Surman, S., Blackman, M. A., and Woodland, D. L. (1996) Major histocompatability complex class II-associated peptides control the presentation of bacterial superantigens to T cells. J. Exp. Med. 183: 1083-1092.

White, J., Herman, A., Pullen, A. M., Kubo, K., Kappler, J. W., and Marrack, P. (1989) The V beta-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and donal deletion in neonatal mice. Cell. 56: 27-32.

Williams, J. (2001). CBRNE—Staphylococcal enterotoxin B. http://www.emedicine.com/.

Wood, A., Todd, C. I., Cockayne, A., and Arbutnott, J. P. (1991) Staphylococcal enterotoxins and the immune system. FEMS Microbiol. Immunol. 3: 121-133.

Yarwood, J. M., McCormick, J. K., Paustian, M. L., Orwin, P. M., Dapur, V., and Schlievert, P. M. (2002) Characterization and expression analysis of *Staphylococcus aureus* pathogenicity island3. Implications for the evolution of staphylococcal enterotoxin pathogenicity islands. J. Biol. Chem. 277: 13138-13147.

Yuh, K., Siminovitch, K. A., and Ochi, A. (1993) T cell anergy is programmed early after exposure to bacterial superantigen *in vivo*. Int. Immunol. 5: 1375-1382.

Zalinskas, R. A. (1997) Iraq's biological weapons. The past as furure? JAMA 278: 418-82.

Zhang, W. J., Sarawar S., and Nguyen P. (1996) Lethal synergism between influenza infection and staphylococcal enterotoxin B in mice. J. Immunol 157: 5049–5060.

Bioterrorism

Edited by Dr. Stephen Morse

ISBN 978-953-51-0205-2
Hard cover, 192 pages
Publisher InTech
Published online 28, March, 2012
Published in print edition March, 2012

This book consists of nine chapters, written by international authorities, discussing various aspects of bioterrorism preparedness and response. Five of the chapters are agent-specific and highlight the pathogenesis, prevention and treatment, and the potential of specific organisms (Rickettsia and Yersinia pestis) or toxins (ricin, botulinum neurotoxins, and staphylococcal enterotoxins) to be used for nefarious purposes. Four chapters discuss different aspects of detecting and responding to a bioterrorism attack. These include methods for spatio-temporal disease surveillance, international laboratory response strategies, detection of botulinum neurotoxins in food and other matrices, and the use of physical methods (ie Raman spectroscopy) to detect spores.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Martha L. Hale (2012). Staphylococcal Enterotoxins, Stayphylococcal Enterotoxin B and Bioterrorism, Bioterrorism, Dr. Stephen Morse (Ed.), ISBN: 978-953-51-0205-2, InTech, Available from: http://www.intechopen.com/books/bioterrorism/staphylococcal-enterotoxins-staphylococcal-enterotoxin-b-and-bioterrorism

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen