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# Staphylococcal Enterotoxin B as a Biological Weapon: Recognition, Management, and Surveillance of Staphylococcal Enterotoxin

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## Abstract

*One of the most important toxin threats in warfare or bioterrorism is Staphylococcal enterotoxin B (SEB), an incapacitating toxin. SEB had been considered and produced as an offensive biologic warfare agent. Staphylococcal enterotoxin B is a toxin associated with incidences of massive food poisoning. The bacteria that produce this toxin (SEB) are universally associated with man and other warm blooded mammals and their spheres of environmental influence include sewage and plumes.* Staphylococcus aureus *can readily be isolated from nose, armpits or anal swabs and about 50% of clinical isolates produce this toxin. Staphylococcal enterotoxin B is one of the superantigens capable of massive nonspecific activation of the immune system including a massive release of cytokines, such as interferon-gamma, interleukin-6 and tumor necrosis factor-alpha. Staphylococcal enterotoxin B is a potential agent of bioterrorism because of the ease of its production and dispersion, a delayed onset of symptoms, an ability to cause high morbidity and the difficulty in discerning between intentional intoxication and natural intoxication when a viable organism is the etiologic agent. This article presents a brief discussion on the recognition, management and surveillance of SEB, as well as the pathogenesis, clinical manifestation, diagnosis, and treatment of patients exposed to this toxin.*

## Introduction

In the 1960s, the USA had an offensive biological warfare program and SEB was one of the agents studied as a biological agent that could be used to incapacitate soldiers in the battlefield. This was an attractive agent because low quantities were required to affect the desired incapacitation when compared with chemicals synthesized in the laboratory (Ulrich et al., 1997).

With the establishment of the Department of Homeland Security after the September 11, 2001 attacks, administrative officers in the Homeland Security have recognized that bioterrorists can use any weapon to carry out their threat. It is important to be mindful of the ordinary symptoms of unusual human and animal diseases and report them to local security authorities as quickly as possible. Delays in recognition and subsequent reporting of bioterrorism can mean the difference in life and death for literally thousands of humans and animals.

Many biological agents and toxins can cause illness in humans, but not all are capable of effecting public health and medical infrastructure on a large scale. The public health infrastructure must be equipped to quickly resolve crises that would arise from a biological or chemical attack. Toxins, chemical compounds synthesized in nature by living organisms, can be classified by molecular weight, source, preferred targets in the body and mechanisms of actions. Many factors place practical limits on their use as mass casualty weapons. These factors include—production, delivery, and environmental stability and host factors (Madsen, 2001). Terrorist use of SEB might be manifested as deliberate contamination of food and water. Therefore the aim of our review is to discuss further the various avenues that SEB could be used as a biological weapon.

## The Toxin

The Staphylococcal enterotoxin B (SEB) comprises a large group of proteins produced by several species of bacteria including *Staphylococcus*, *Streptococcus* and *Mycoplasma* (Bergesll, 1970 & 1979). Staphylococcal enterotoxin B is responsible for a number of extensive pathophysiological changes in humans and mammals and triggers an excessive cellular immune response leading to toxic shock (Kaempfer, 2004). Staphylococcal enterotoxin B, together with ricin and epsilon toxins, is classified as category B Priority Pathogens by National Institute for Allergy and Infectious Diseases (CDC, 2000).

*S. aureus* are found in all foods that have been handled by humans or that have been contaminated by animal matter. They grow well in most prepared food, including meats, vegetables, fruits, pastries, and milk prod-

ucts (Vela, 1997). In the laboratory, *S. aureus* grows well on nutrient agar containing about 10% sodium chloride. *Staphylococcus aureus*, with *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and enteropathogenic *Escherichia coli* are responsible for more than 90% of food poisoning cases each year in USA (Vela, 1997). Exotoxins (proteins) and related pyrogenic toxins from these bacteria easily diffuse out of the cell (Novick et al., 2001). A distant related protein to SEB, toxic shock syndrome toxin-1 (TSST-1), also produced by *Staphylococcus aureus*, was isolated in the early 1980s and is responsible for the induction of tampon-related toxic shock (Novick et al., 2001). Staphylococcal enterotoxin B is a part of a set of exotoxins produced by *S. aureus* which comprise about 15 antigenically distinct proteins and include the following: SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, SEH, SEG, SEI, SEJ, SEK and the last one discovered recently was identified as SEU (Lefertre et al., 2003). Many of these toxins are closely related and are collectively called superantigens because they interact with the immune system to activate a very high percentage of T-cells (Miehke et al., 1992).Various studies have shown that not all these toxins play a role in food poisoning (Lefertre et al., 2003). According to the information provided, all but two SE's cause gastrointestinal (GI) symptoms (Su & Wong, 1995, McLauchlin et al., 2000; and Omoe et al., 2002).

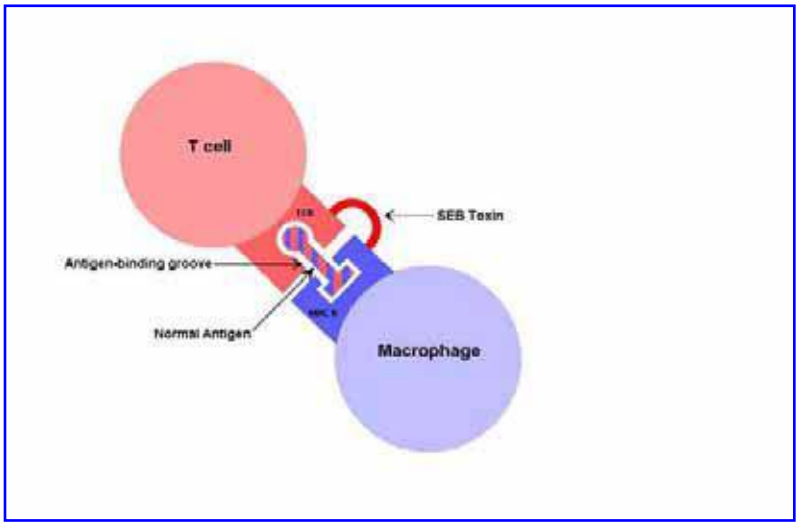
Staphylococcal enterotoxin B could pose a great risk to consumer health and can be classified as a low molecular protein (24 - 29KD) (Su & Wong, 1997). Because of low molecular weight, SEB could induce gastroentric symptoms which include diarrhea and vomiting in humans, and as superantigens, may also cause toxic symptoms (Marreck & Kappler, 1990) by initiating the activation and proliferation of T-cells with certain Vβ (variable domain of T-cell receptor β-chain) regions on their T-cell receptor (Miehke et al., 1992). If mature animals are exposed to SEB, mature T-cells bearing target Vβ's respond to the challenge by rapid proliferation and production of cytokines (Kotsin et al., 1993). SEB Symptoms can be induced by as little as 30ng (Kotsin et al., 1993). Experiments have shown that for aerosol exposure, the effective dose, or ED50 (dose capable of incapacitating 50% of the exposed human population), is 0.0004mcg/kg, and the lethal dose, or LD50, is .02mcg/kg (Rusnak et al., 2004). The extremely small amount of material that is required for toxic effect indicates that a complex is necessary for the toxin to exert its effect. SEB can represent a practical bioterrorist weapon because purified toxin can be isolated from *S. aureus* culture supernatants.

## Mechanism of Action

Staphylococcus enterotoxin B must first enter the body and gain access to immune cells to do any harm. SEB binds to major histocompatibility complexes (MHC) class II molecules and stimulates T-cells by binding to T-cell antigen receptors with strong avidity, independent of antigen recognition (Figure 1). Up to one in five T-cells may be activated, whereas only one in 10,000 is stimulated during a usual antigen presentation. When these T-cells are stimulated, an immediate activation and prolif-

**Figure 1**

Superantigens and the non-specific stimulation of T-cells: Superantigens (SEB) bind directly to class II major histocompatibility complexes (MHC II) of antigen-presenting cells outside the normal antigen binding groove. Up to one in five T-cells may be activated. Cytokines are released in large amounts, causing the symptoms of toxic effects of SEB. Figure by C. Alexander Designs. All rights reserved.



erations of the T-cells with Vβ's variable domain of T-cell receptor will ensue (Kappler et al., 1989). When exposed to SEB, mature T-cells bearing target Vβ's (variable domain of T-cells receptor β-chain) respond to the challenge by rapid proliferations and productions of cytokines which are thought to mediate most of the toxic effects of SEB (Marrack et al. 1990, Stiles et al. 2001). So engagement of class II molecules by the toxin on macrophages or mast cells stimulates these cells and causes release of soluble mediators beneficial to the host in small quantities. SEB binds directly to class II major histocompatibility complexes of antigen-presenting cells outside the conventional antigen-binding grove. This complex recognizes only the Vβ element (variable domain of T-cells receptor β-chain) of the T cell receptor. Thus, any T cell with the appropriate Vβ element can be stimulated, whereas normally, antigen specificity is also required in binding (Kotzin et al., 1993).

## Symptoms

Symptoms of SEB intoxication include a sudden onset of fever, about 40°C to 41°C, chills, headache, myalgia, and a non-productive cough. Some patients may develop shortness of breath and chest pain. Fever may last 2-5 days and cough may continue for up to one month. Patients may also present with nausea, vomiting, and diarrhea when the toxin is swallowed (Ulrich et al., 1997). The effects on those exposed while under stress, such as soldiers in combat situations, may appear to be much more severe. This exposure can result in vasodilation and pathological drop in blood pressure, respiratory distress, shock and death within 40-60 hours of exposure. Some forms of SEB-intoxication result from absorption of the toxin into circulation from mucosa surfaces (gut) (Ulrich et al., 1997). In a recent finding, individuals working in a laboratory were diagnosed with conjunctivitis with periocular or facial swelling as a result of ocular or cutaneous exposure. This was the first report of eye irritation involving SEB. This emphasizes the importance of face masks and eye protection for those individuals working with SEB (Rusnak et al., 2004). Although SEB is not generally considered lethal, high levels of exposure can lead to septic shock and death.

## Pathogenesis and Clinical Manifestation

Staphylococcal enterotoxin B symptoms occurring in humans is associated with the site of entry. When the toxin is ingested, this will result in the inflammation of the gut leading to diarrhea and vomiting. If the toxin is absorbed through the dermis, there is an inflammation of the skin resulting in dermatitis and delayed type hypersensitivity (DTH) (Rusnak et al., 2004). However, when the eye is infected, there is an inflammation of the eye resulting in iritis. In inhalation of SE, there is a sudden onset of fever, headache, chills, myalgia and a non-productive cough. In more severe cases, the patient may develop dyspnea and retrosternal chest pain leading to the inflammation of the lung and respiratory distress (Ulrich et al., 1997). When the toxin is absorbed into the circulation, there is inflammation of the vasculature resulting in toxic shock (McLauchlin et al., 2000). Two hours after intoxication, patients with SEB typically begin to experience blurred vision, headache, abdominal distress, diarrhea, and vomiting and generalized body weakness (Rusnak et al., 2004). Medical treatment is not prescribed for SEB intoxication unless there is excessive loss of electrolytes from vomiting and diarrhea. In this case, electrolyte replacement and treatment of symptoms are the only measures indicated.

## Epidemiology

The unfortunate fact remains that humans are often the most sensitive detector of a biological attack (Ulrich et al., 1997). Without the knowledge of the attack, an increased number of patients presenting with signs and symptoms caused by the disseminated disease agent is the first indicator that SEB exposure has occurred. SEB is not contagious and cannot be transmitted from person to person. In contrast, when this toxin is expressed in *E. coli*, the toxin produced is very potent (Kotzin et al., 1993). No instances of waterborne toxin contamination have ever been reported, although the potency of SEB has led to speculation that it might be used to contaminate a municipal water supply. If food were deliberately contaminated and used as a carrier, the outbreak would need to be distinguished from naturally-occurring foodborne Staphylococcus food poisoning. Staphylococcal food poisoning is quite common in the USA and all other countries of the world. Outbreaks are numerous during all seasons of the year, with a noticeable increase during the summer months. The rapidity of onset and severity of SEB intoxication depends on the rate and amount of toxin absorbed. When the toxin is ingested through food, symptoms may begin as soon as two hours after ingestion (Kotzin et al., 1993). Symptoms may last as long as 12 hours then disappear completely. Normally, recovery is uneventful and no residual effects remain even after severe intoxication. Rusnak et al., reviewed occupational exposure to SEB and concluded that the knowledge of full clinical spectrum of SEB intoxication is important to healthcare workers evaluating persons with potential exposure to SEB and including in the context of bioterrorism (Rusnak et al., 2004). Any outbreak of SEB should bring to mind the possibility of bioterrorism, but certain features would be particularly suggestive such as multiple simultaneous outbreaks with no common source (Rusnak et al., 2004).

## Detection of SEB

Methods for fast detection and identification of SEB are highly desired to provide early information to healthcare providers and safety officers in the event of a bioterrorist attack. In recent years, rapid progress has occurred in the area of biosensor development. Should SEB be used as a bioterriorist agent, one should expect exposure by inhalation or contamination of food and water. On the battlefield, where the SEB will be distributed as aerosol, the device will have specific detection needs. For example, this device should be automatic, unattended, and remote or carried as an analytical test system to be used under battlefield conditions. In this context, the device could be used to analyze such samples as air, water, personnel and equipment. Menking & Goode 1993 described the detection of SEB using the light addressable potentiometer sensor (LAPS). Using this method, a lower limit of SEB (2ng/mL) was detected. SEB has also been detected with an impedance-based immunobiosensor (DeSilva et al., 1995). Tempelman et al., (1995) reported the use of a fiber optic biosensor for the detection of SEB on a variety of clinical, environment and military samples. King et al., 1999 used the Man-portable Analyte Identification System (MANTIS) which is the first fully automated, self-contained, portable fiber optic biosensor for the detection of SEB. This device detected SEB spiked into liquid samples with no false positives and could perform simultaneous immunoassays rapidly in the field with little or no intervention by the user. Homola et al., 2001 have reported the use of Surface Plasmon Resonance (SPR) which is a wavelength modulation-based sensor to detect SEB in milk. This sensor was able to detect SEB at low concentration of 5ng/mL without amplification. This report also indicated that SPR could be tailored for the detection of various food pathogens. Over the past few years, multiple PCR assays and multiplex PCR assays which detect specific gene sequences for SE's and TSST-1 by DNA amplification have been developed (McLauchlin et al., 2000, Schmitz et al., 1998, Sharma et al., 2000). This real-time PCR appears to be a much more efficient method because it allows for the analysis of large number of samples at the same time, thereby saving more time than the conventional PCR and does not detect any false positives. Finally, 24 hours after exposure to SEB, the toxin could be identified from nasal swabs from individuals exposed by aerosol. This may be important in the battlefield since this can be used as an early diagnosis.

## Biosafety and Decontamination

With increased funding for biodefense research and many institutions working with SEB, it is possible there will be an increase in laboratory exposure and intoxication with SEB. It is necessary to document the symptoms of SEB intoxication in order to educate healthcare workers and safety officers to enable them to properly identify those individuals at risk and thereby prevent exposures to SEB. Biosafety is the measure intended to prevent accidental release of SEB from a research facility that could endanger the public and environment. Biosafety is achieved through use of primary and secondary containment devices such as Biological Safety Cabinets (BSC), good laboratory practice/technique and glove boxes. These barriers protect the researcher from the toxin (SEB) while the filters prevent the toxin (SEB) from entering the environment. In a report addressing the issue of biological safety cabinets' (BSC) efficacy, a contractor noted that individuals working with SEB on laboratory benches without BSC experienced toxic reactions (Wendum, 1996). Staphylococcal enterotoxin B is a biotoxin that can be acquired by inhalation, ingestion, or injection. The toxin is a highly-soluble protein that is easily removed with soap and water and inactivated by autoclaving. After exposure to SEB, clothing, skin, and eye should be washed thoroughly with soap and water for at least 15 minutes. Contaminated surfaces (for example laboratory tables and BSCs) should be cleaned with disinfectant solution and contaminated objects secured and autoclaved.

## Biosecurity

The Centers for Disease Control and Prevention (CDC) and Animal Plant Health Inspection Service (APHIS) have provided a model list that could be followed by facilities as a basis for biosecurity standards (42 CFR, Part 73.11). In this list, the CDC has grouped agents of bioterrorism into three categories (A, B, and C) depending on their impact on public health and environment. Staphylococcal enterotoxin B is a category B agent, which is moderately easy to disseminate, and, if exposed within the civilian population, will result in moderate morbidity and low mortality rates. Most of these agents in category B that could be used for bio-terrorist acts may be obtained from sources such as patients and infected animals. Therefore, a terrorist could have access to these sources and be able to isolate the agents in order to use them as a weapon of mass casualty. The probability of a terrorist having the technical expertise and skills required to isolate and culture these organisms is very low. The greater risk would be the terrorist stealing the agent from a research laboratory or purchasing the agent from a national culture collection or a commercial supplier under false pretense. The essential provision of a biosecurity measure is to make it difficult for the terrorist to acquire these agents, ensuring that researchers are performing legitimate research, and research facilities are off limits to individuals who have not gone through FBI security screening. The biosafety regulation determines who has

access to the agents, what agents (SEB) the entity possesses, and the locations of the facilities using these agents (CFR 42 Part 73). Therefore, any facility that has acquired these agents (SEB) is required, by regulation, to conduct threat, vulnerability, and risk assessment. Since it is difficult to obtain a quantitative accounting of all the agents of bioterrorism, it is important to develop a security plan tailored specifically to the agent in use (in this case SEB) and the unique characteristic of the agent (ABSA, 2002). Any security plan should reference the CDC revised and expanded guidelines entitled "Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents" (Richmond et al., 2002). Other forms of security required by the regulation include inventory controls in order to track internal possession and transfers, and inactivation and disposal of culture after use. Physical security is designed to impede unauthorized entry into the laboratory in order to steal the select agent (SEB) stored within the facility. This security should be at the level of hazard or threat associated with the agent (in this case SEB). In order to provide security and protection for facilities researching with SEB, all the guidance provided above needs to be followed precisely.

## Treatment

The American experience on September 11, 2001 has accelerated the demand for the development of therapies and vaccines against various agents of bioterrorism. Treatment for SEB is administering the victim supportive medical care to minimize the effect of the intoxication. The supportive medical care would depend on several factors, such as the route by which the victims were poisoned (that is, either by inhalation, ingestion, or skin or eye exposure). Medical care needed at this time may include helping the victim breathe, giving the victim intravenous fluid and flushing their stomach. For oral exposure, washing out mouth with water and if swallowed, vomiting should be induced. Historically, SEB vaccine research which focused on formalin-inactivated toxin (Silverman et al., 1969, Tseng et al., 1993) has been carried out by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). This vaccine, produced by prolonged incubation in formalin, was evaluated for its ability to induce protective antibodies in monkeys by intracutaneous (Silverman et al., 1969), intramuscular (Tseng et al., 1993), intratracheal (Tseng et al., 1995), and intragastric (Tseng et al., 1995) routes. This vaccine was shown to be immunogenic when delivered parentally with no clinical side effects and stimulates protective antibody responses (Silverman et al., 1969). However, SEB toxoid is a poor immunogen when given nasally but, in combination with protosomes and biodegradable microspheres, it stimulates antibody responses. USAMRIID abandoned this vaccine despite its good attributes in favor of a recombinant vaccine which uses a site-directed mutant (Boles et al., 2003, Boles et al., 2003). These vaccines were designed with the knowledge of molecular interactions between SEB and MHC II/Vβ TCR. Mantis (2005) has indicated that a vaccination regimen for humans should be carefully optimized since immunized animals (5μg/dose) showed lower rate of survival. In an experiment in which mice were immunized orally or intranasally with SEB triple mutant vaccine in combination with cholera toxin as adjuvant, anti-SEB IgA antibodies were stimulated in serum and salivary secretions (Stiles et al., 2001). Research in recombinant vaccine will ultimately lead to the development of safe, effective vaccines that can be distributed through oral, intranasal or transcutaneous routes which will be capable of inducing both systemic and local immunity.

## Conclusion

The use of SEB as a weapon of mass casualty is considered likely for several reasons, mainly high morbidity with ease of production and dispersion, the delayed onset of disease symptoms associated with high morbidity and low mortality and difficulty in diagnosis. Staphylococcal enterotoxin B is a superantigen capable of massive nonspecific activation of the immune system. Because of the remarkable toxicity and stability, they would most likely be disseminated as an aerosol, in food, or water supplies. Several vaccine trials in animal models appear to be promising but, in order to perform these trials in human subjects, it will be necessary to understand which receptors are used to attach and penetrate the epithelial barrier, the effects of SEB on mucosal cells and role of mucosal immunity. In the context of bioterrorism, this review will be relevant to military personnel considering that SEB is an incapacitating biowarfare toxin.

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## Fact Sheets on Terrorist Attacks

The U.S. National Academies of Science has prepared fact sheets to provide reporters with reliable information on biological, chemical, nuclear, and radiological attacks. This effort was a collaboration with the U.S. Department of Homeland Security, and the Radio and Television News Directors Foundation. ABSA members may find the information useful in educational efforts on emergency planning.

The fact sheets can be found at www.nae.edu/factsheets.

**Biological Attack** (pdf file, 277 KB)—Where do biological agents originate? What's the difference between "infectious" and "contagious"? How long after exposure will symptoms appear?

**Chemical Attack** (pdf file, 72 KB)—What are the different origins of toxic chemicals that could be used? How do chemical toxicities vary? What are the practical steps to take if there's a chemical release?

**Radiological Attack** (pdf file, 68 KB)—What are radiological dispersal devices, a.k.a. "dirty bombs"? How are they different from nuclear bombs? What are their physical and psychological health effects?

**Nuclear Attack** (pdf file, 192 KB) **NEW!**—What is radioactive fallout, and how is it dangerous? What are the short-term and long-term effects of radiation exposure? What is the likely size of a nuclear explosion from an attack by terrorists?