HEALTH EFFECTS OF PROJECT SHAD BIOLOGICAL AGENT:

STAPHYLOCOCCAL ENTEROTOXIN TYPE B

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SPECIAL NOTE ON PSYCHOGENIC SEQUELAE OF PERCEIVED EXPOSURE TO BIOCHEMICAL WARFARE AGENTS

This report deals primarily with the biological health challenges engendered by the agent that is the subject of the report. Nevertheless, this report also incorporates, by reference and attachment, a supplement entitled "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents".

The supplement addresses and describes a growing body of health effects research and interest centered upon the psychogenic sequelae of the stress experienced personally from actual or perceived exposure to chemical and biological weaponry. Because awareness of exposure to agents in Project SHAD logically includes the exposed person also possessing a perception of exposure to biochemical warfare agents, the psychogenic health consequences of perceived exposure may be regarded as additional health effects arising from the exposure to Project SHAD agents. This reasoning may also apply to simulants and tracers. Therefore, a general supplement has been created and submitted under this contract to address possible psychogenic effects of perceived exposure to biological and chemical weaponry.

Because such health effects are part of a recent and growing public concern, it is expected that the supplement may be revised and expanded over the course of this contract to reflect the actively evolving literature and interest in the issue.
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I. EXECUTIVE SUMMARY

Staphylococcal Enterotoxin Type B (SEB) is one of at least 17 enterotoxins produced by the common infectious pathogen, *Staphylococcus aureus*. SEB is a heat-stable 28-kilodalton protein toxin. Unlike many other enterotoxins, SEB can cross epithelial and mucosal tissue intact. Its stability, toxic properties, and ability to be easily aerosolized make it an attractive biological weapon. SEB was part of the American biological weapon stockpile until the 1970s and was formally defined as a biological warfare agent in the 1972 Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction.

Biologically, SEB acts as a superantigen, activating the immune system at picomolar concentrations. The toxin activates both T-lymphocytes and antigen presenting cells (APC) by crosslinking the class II Major Histocompatibility Complex (MHC) on the APC to the Vβ chain of the T-cell receptor. These interactions result in the polyclonal activation of T-cells (predominantly a Th-1 response) along with the release of various cytokines (interleukin-2 (IL-2), interferon-gamma, interleukin-6 (IL-6)) and tumor necrosis factor–alpha (TNF-α) and chemokines (pulmonary and activation-regulated chemokine (PARC), MIP-1alpha, MIP-1beta, and MCP-1). Approximately 20% of all CD4+ T-cells can be activated by SEB as compared to 1 in 100,000 to 1,000,000 that are activated by a typical peptide antigen. Both CD4+ (helper) and CD8+ (cytotoxic) T-cells that express Vβ7 and Vβ8.1, 8.2 and 8.3 T-cell receptor chains (TCR) can be activated by SEB. In addition to activating T-cells, SEB exposure can induce anergy or unresponsiveness in memory T-cells and apoptosis in cells that initially proliferate. This can prevent the immune system from responding to pathogens and may be a mechanism by which *Staphylococcus aureus* is able to evade the immune system.

The oral route of exposure is the best-known means of producing SEB-induced illness. Staphylococcal enterotoxins are common causes of classic food poisoning. The enterotoxic effects of SEB, the ability to produce nausea and emesis, appear to be distinct from its ability to stimulate T-cells. Nonetheless, the aerosol dispersion of SEB can be used as a weapon in military or bioterrorist actions. Because SEB intoxication is rarely fatal, its use is likely to be limited to inducing enemy incapacitation for a brief strategic period, rather than for inflicting large-scale mortality. The onset of action is usually 1-6 hours after exposure and as little as 1 microgram of SEB can cause enterotoxic effects in adults.

Diagnosis can be difficult because by the time SEB’s effects appear, the toxin has been cleared from the serum. Conclusive diagnosis of SEB intoxication is nevertheless most properly made through the use of enzyme-linked immunosorbent assays (ELISA) of tissues, body fluids, or environmental samples. Urine samples can be helpful for rapid diagnosis as the toxin may be discernibly present in less than one day of exposure. Nasal swabs similarly may yield positive results within 12-24 hours after exposure.
The clinical recognition of SEB intoxication can be difficult because of the general nature of the initial symptoms (e.g. fever, myalgia, nausea). Other toxins or agents causing nausea and vomiting must be ruled out, particularly the heat-stable toxin of *Bacillus cereus*. Intoxication with metals or nitrates can also yield similar symptoms. In the very early stages following SEB exposure when intense fever is prominent, distinguishing SEB intoxication from inhalation anthrax, tularemia, plague, or Q fever can be problematic in a biowarfare context.

The most commonly observed acute effects of SEB exposure are two syndromes that vary according to the main likely routes of exposure -- oral and inhalational. (These, however, are not the only possible routes as SEB dermatitis from prolonged skin exposure has been demonstrated. SEB has been shown to contact and enter the body through *S. aureus* colonization of skin, wound infections, and feminine hygiene devices.)

The symptoms of oral ingestion of SEB are the well-observed effects of food poisoning. There is a sudden onset of nausea a few hours after food consumption, which is followed by vomiting, cramping abdominal pain, and watery unbloody diarrhea. Anorexia and dehydration are frequent. Fever is less common (about 25% occurrence) and pulmonary involvement is not associated with oral exposure. Tachycardia, hypotension, hyperperistalsis, and a diffuse nonlocalizing abdominal pain are also possible symptoms. The symptoms can incapacitating but usually resolve quickly, even within 24 hours.

Inhalation exposures are more complex and last longer, generally one to two weeks. Symptoms usually manifest within a few hours of exposure. Symptoms of inhalation exposure typically commence with a fever that can run as high as 40 deg C. Myalgia, headache, chills, chest pain, rales, dyspnea, and a cough (usually nonproductive) tend to follow. Nausea and vomiting may also occur following exposure but diarrhea has not been reported.

Death is uncommon in acute cases. Evidence from animal testing and human tissue suggests that ingested SEB may also be a causative factor in sudden infant death syndrome (SIDS). Relapse or recurrence is not reported for acute episodes except in rare cases of nonmenstrual toxic shock syndrome, where persistence of an *S. aureus* colony along with an absence of seroconversion explains the renewed effect of SEB a few days or weeks after an initial acute episode is resolved.

Though death is rare, severe and even fatal septic shock, including nonmenstrual toxic shock syndrome, are possible consequences of exposure to large dosages. High pulmonary doses may also cause chest pain, pulmonary edema, and an adult (or "acute") respiratory distress syndrome (ARDS). A common respiratory sign is patchy interstitial edema on radiologic examination.

In terms of long-term effects, SEB exposure has been increasingly implicated in the genesis and exacerbation of certain allergic diseases like atopic dermatitis, psoriasis vulgaris, vernal keratoconjunctivitis, and atopic keratoconjunctivitis. SEB has also been
implicated in the induction of autoimmune diseases such as Graves disease, arthritis, and even multiple sclerosis (MS). In a rare case, SEB exposure has been associated with a long-term elevation of liver function tests though the role of SEB exposure was deemed inconclusive.

Because there is no antitoxin, general supportive care – supplemental oxygen, hydration, pain control -- for the term of the illness is the standard recourse for those afflicted by SEB. Protective masks are recommended as a measure to prevent against inhalation exposure. Decontamination is usually performed with a solution of sodium hypochlorite. A promising inactivated recombinant SEB vaccine is in development and has been tested on primates. Compounds known to inhibit TNF-α production, such as Pirfenidone, niacinamide and pentoxifylline, have been shown to be effective in blocking both the immunological and toxic effects of SEB in cells and animals.

Psychogenic effects specific to SEB are not reported. (General psychogenic effects of perceived exposure to agents of chemical and biological warfare are examined in the supplement “Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents.”) Secondary literature is fairly comprehensive and consistent on the subject of SEB but the association of SEB exposure with chronic allergic diseases, autoimmune disorders, and sudden infant death syndrome are not treated in general discussions of the toxin as a warfare agent.
II. BACKGROUND DATA

Identification & Biochemistry

Names: Staphylococcal enterotoxin type B; Enterotoxin B, staphylococcal; SEB

CAS registry number: 39424-53-8

Protein sequence: esq pdpkdelhk mskftglmmd sainvksidq flyfdliysi kdtklngynv vrvfknkdl adkydkyvd vfganyqyc yfskktndin shqtldrrknc myngvtehng nqldkrysir vrvfedknl lsfdvqtnkk kvtaqeldylv trhylvnnkk lyefnspye tgyikfiene nsfwydmmpa pgdkfdqsky lmmyndkmv dskdvkievy lttkkk

Staphylococcal enterotoxin type B is commonly identified by the abbreviation SEB. (In U.S. military use, it also bore the code names PG, PG2, and, in Project SHAD, SE). SEB is an exotoxin (externally secreted poison) produced by Staphylococcus aureus, the common bacterial pathogen whose effects go by the popular informal designation “staph infection”. S. aureus is a Gram-positive clump-forming coccus that frequently colonizes nasal passages and axillae (Ulrich et al. 1997; Williams 2004).

SEB is one of at least seventeen different enterotoxins that can be produced by Staphylococcus aureus (Sergeev 2004). The designation “enterotoxin” derives from the fact that the Staphylococcus toxins act with a particularly intense toxicity on the gastrointestinal tract, often inducing both emesis and diarrhea (Williams 2004). SEB is among the most common causes of ordinary food poisoning along with botulin toxin and the trichothecene mycotoxins (E-medicine 2004).

SEB has a molecular weight of about 28 kilodaltons. It is made up of 239 amino acid residues. SEB is a very stable protein and readily soluble in water. It can withstand changes in temperature, including several minutes of boiling. It can be stored for over a year in a freeze-dried state (Ulrich et al. 1997; Williams 2004).

SEB is commonly described as a "superantigen" and more precisely a “PTSAg”, a pyrogenic (i.e. fever-inducing) toxin superantigen. A superantigen is a compound capable of stimulating an unusually large number of T-cells. Members of the PTSAg category include other staphylococcal enterotoxins (A, C, D, E, G, H) and TSST-1 (Toxic Shock Syndrome Toxin-1) (Dinges 2000). Unlike most other enterotoxins, SEB appears to be able to efficiently penetrate epithelial tissue by receptor mediated transcytosis. This means that even local exposures to SEB can result in systemic exposure through the blood (Hamad 1997).
SEB, like most enterotoxins, has both enterotoxic and immunostimulatory properties. Both mutations and chemical modification studies indicate that these properties are independent of one another. An SEB-F44S mutant was found to retain emetic activity even though it had lost its ability to stimulate T-cells. The mechanism for emesis is currently not known, but T-cell stimulation results from the bridging of MHC class II molecules and the TCR Vβ chains (Harris et al. 1993).

SEB’s binding to both MHC class II molecules and the TCR Vβ chains has been well studied. SEB has been shown to bind to and activate both CD4+ and CD8+ T-cells that express either the Vβ7 and Vβ8.1, 8.2 and 8.3 TCR chains. This typically results in approximately 20% of CD4+ T-cells responding to SEB. This contrasts with a rate of only 1 in 100,000-1,000,000 cells responding to a typical peptide antigen (Herman et al. 1991). Surprisingly, the affinity of SEB to both types of molecules is only in the micromolar range. Given the observation that SEB is able to activate the immune system at picomolar concentrations, authors have postulated a role for the CD4 receptor and/or the TCR-α chain in forming a high affinity complex between TCR Vβ, HLA-DR and SEB. Crystal structures are available for both SEB:HLA-DR1 and SEB:TCR β complexes. The SEB:HLA-DR1 structure indicates that SEB is not dependent upon, nor interferes with, peptides binding to MHC molecules. The SEB:TCR β structure shows that SEB binds to the complementarity-determining region (CDR2) of the TCR β chain. The TCR binding site on SEB is in close proximity to the HLA-DR1 binding site indicating that binding to the two receptors is not independent of one another (Li 1999).

**Use as Weapon**

SEB was part of the US biological weapons stockpile until its final destruction and banning in 1972. SEB was classified as a biological, rather than chemical, warfare agent in the 1972 Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. It is currently classified as a "category B" biowarfare agent along with brucellosis, cholera, Q-fever, and ricin (Williams 2004).

SEB’s ability to affect and incapacitate multiple human physiological systems is a key factor in its attractiveness as a military tool. SEB is also stable, and readily and efficiently aerosolized. It is estimated that SEB would cause deleterious health effects on up to 80% of those exposed by an aerosol method. These factors, along with the observation that relatively small quantities are needed to inflict injury in a reasonably short time period, make SEB an agent of interest in biowarfare (Williams 2004; Ulrich et al. 1997).

Because SEB is far more likely to produce a period of illness rather than death, SEB is considered an incapacitating biowarfare agent, rather than a lethal one. Nevertheless, its effective use on an active battlefield could be quite valuable for neutralizing the fighting
ability of opposing forces. Additionally, terrorists may be able to insert SEB into the food supply, or deliver it by aerosol to a populated area, and incapacitate a large part of the population.
III. INCIDENCE, PATHOGENESIS & DIAGNOSIS

It is difficult to determine the precise incidence of SEB intoxication in the general population. That is because food poisoning is often untreated and diagnoses of SEB-induced illness are usually based on unconfirmed and unreported clinical observation. Many conditions that manifest with gastrointestinal disorders can be difficult to distinguish from SEB-induced conditions. (Williams 2004) (Differential diagnoses for SEB intoxication are provided in the "Diagnosis" subsection, further below.)

**Incubation**

The most common means of human intoxication is consumption of improperly stored food that has been colonized by toxin-producing bacteria. Incubation of SEB usually ranges from 1-8 hours after first exposure, and occasionally up to 18 hours. Inhalation exposure has an incubation range typically of 1-6 hours. (Williams 2004)

**Pathogenesis**

The pathogenesis of enterotoxin intoxication continues to be a subject of intensive study (Ide 2004; Lamont 2002; Kim 2003). The nature of the toxin's action remains insufficiently understood. Even the process by which SEB causes emesis is not fully clear although the characteristic gastrointestinal symptoms that result from oral ingestion are believed to involve the release of histamine and leukotrienes from mast cells (Williams 2004).

The pathogenic effects of aerosol exposure believed to be primarily mediated by the marked stimulation of T-lymphocytes. SEB bridges the major histocompatability complex (MHC) class II proteins on antigen presenting cells to the T-cell receptor, which in turn causes intense T-lymphocyte stimulation and proliferation (Izeradjene 2001). Massive production and secretion of various cytokines (interferon-gamma, interleukin-6 and tumor necrosis factor–alpha) and chemokines (MIP-1alpha, MIP-1beta, PARC and MCP-1) follow SEB exposure (Ulrich et al. 1997; Krakauer 1999). Antibodies generally develop within 6 days of exposure (Ulrich et al. 1997; Williams 2004). Animal studies have indicated that activated T-cells resulting from intratracheal administration of SEB can induce interstitial pneumonia in mice (Fujiki 1999).

In addition to activating T-cells, SEB is capable of inducing anergy or unresponsiveness, predominantly in memory type T-cells (Rellahan et al. 1990). Anergy is believed to result from the repeated partial signaling that results from SEB binding and the lack of costimulatory signals (Heeg et al. 1995). In addition to anergy, clonal deletion of proliferating T-cells can occur following SEB exposure. SEB is particularly effective in inducing apoptosis of Th1 cells mediated by Tc1 cells. Therefore, in contrast to most activation-induced cell death that is suicidal, SEB induces fratricidal apoptosis of Th1 cells (Gorak-Stolinska et al. 2002). Although an initial injection of SEB activates T-
cells, repeated injections appear to induce unresponsiveness mediated through the
differentiation of CD4+ T-cells into suppressor cells that express high amounts of the
immuno-inhibitory molecule CTL4-A (Noel et al. 2001). Whether SEB exposure results
in long-term immune activation or tolerance is determined by a multitude of factors
whose interactions between one another are difficult to predict.

**Diagnosis**

As a practical matter, diagnosis of SEB intoxication is normally clinical and
epidemiological. Conclusive diagnosis of SEB intoxication is most properly made
through the use of enzyme-linked immunosorbent assays (ELISA) of tissues, body fluids,
or environmental samples. Sources for toxin analysis include nasal swabs taken less than
24 hours after aerosol exposure, induced respiratory secretions, and blood (in tiger-top or
red top tubes). Toxin presence in serum is usually transient but it will accumulate in urine
for several hours after exposure. Antibodies may not appear for several days (Williams

Physical examination in SEB intoxication may be unremarkable. Chest X-rays are often
normal but in more serious cases after respiratory exposure, pulmonary edema or a
presentation of adult respiratory distress syndrome (ARDS) may be seen (Williams
2004).

In cases where the toxin is derived from a bacterial infection, and cultures can be
obtained, detection of even minute strains of the toxigenic pathogen can be performed
within 24 hours of exposure by (1) polymerase chain reaction (PCR) amplification and
(2) toxin gene–specific oligonucleotide primers. (Ulrich et al. 1997)

**Differential Diagnoses**

Differential diagnoses of inhalational SEB include the following conditions and toxic
agent exposures:

Cholecystitis, biliary colic, gastritis, peptic ulcer disease, gastroenteritis, giardiasis,
pancreatitis, pericarditis, cardiac tamponade, adult respiratory distress syndrome,
scorpion envenomations, hypovolemic shock, large and small bowel obstruction,
cyanogens chloride, chlorine, chloropicrin, diphosgene, phosgene, Teflon, tabun, sarin,
VX, VX2, Ricin, and mustard vesicants (Williams 2004).

In very early clinical stages, i.e., when fever is present, distinguishing SEB intoxication
from inhalation anthrax, tularemia, plague, or Q fever may be problematic. SEB fever
tends to dissipate quickly rather than progress for an extended period (Williams 2004,
IDEAS, Ulrich et al. 1997).
Differential diagnoses of ingested SEB toxin include all other toxins causing nausea and vomiting, particularly the heat-stable toxin of *Bacillus cereus*. Intoxication with metals or nitrates can also yield similar symptoms (IDEAS 2004).
IV. HEALTH EFFECTS

Overview

As noted in the previous sections, there are essentially two main types of systemic exposures to SEB: ingestion (classic food poisoning) and inhalation. This report on SEB’s health effects will treat both, but the acute exposure section will concentrate more upon the inhalation effects following aerosol exposure. The effect of direct contact of SEB on the skin or through percutaneous pathways is also discussed. Suspected long-term effects from SEB exposure, including autoimmune, dermal, ocular, and upper respiratory allergic diseases, are also reported. The nature and causal role of SEB in these diseases are not firmly established. Animal studies, epidemiological associations and suggested pathobiological explanations suggest that SEB exposure can play a role in either the initiation or exacerbation of a variety of diseases.

SEB typically engenders a self-limited acute debilitating illness that is rarely fatal, at least in the presence of adequate hydration. In aerosol exposures, the human ED$_{50}$ has been estimated to be 0.0004 µg/kg, and the human LD$_{50}$ has been estimated to be 0.02 µg/kg (Ulrich et al. 1997). Recovery from all forms of exposure is usually complete within two weeks with some respiratory effects (e.g. non-productive cough) possibly lasting for a month. Nevertheless, higher exposure to SEB may lead to septic shock and death (Williams 2004). Some studies also suggest that sudden infant death syndrome (SIDS) is a consequence of ingested enterotoxin septic shock (Kamaras 2001a).

Although SEB often has little effect in mice by itself, laboratory studies indicate that endotoxin from Gram-negative bacteria acts in synergy or potentiates the toxicity of enterotoxins. A mouse can become severely affected by even nanograms of SEB when Gram-negative bacteria endotoxin is also administered (Ulrich et al. 1997).

The route of exposure can determine the acute symptomatology of SEB. Fever, for example, is less common when SEB is ingested (25%), while pulmonary manifestations usually only occur after exposure through the respiratory pathway. Diarrhea is typically only seen following oral exposure (Ulrich et al. 1997, IDEAS 2004, Williams 2004).

There is no literature reporting any direct relapse of an acute episode of SEB exposure, with the exception of reports of a nonmenstrual toxic shock syndrome (TSS) from SEB during which recurrence within days or weeks is possible (Andrews 2001; Crass 1985). One case study, detailed below, also found a long-term multi-year mild elevation in patient liver function tests but the connection of that to SEB exposure is equivocal (Ulrich et al. 1997).

General biologic signs of SEB intoxication include neutrophilic leukocytosis and an elevated erythrocyte sedimentation rate (Williams 2004).
Signs & Symptoms: Acute Dermal/Percutaneous

Dermal exposure commonly arises through the presence of *S. aureus* on the skin. A test of dermal exposure to SEB (occluded patch) found that the toxin acutely elicited dermatitis in both healthy and allergy-sensitive individuals (Strange 1996). SEB from *S. aureus* infections in surgical wounds and certain vaginal contacts has been implicated in a recurrent nonmenstrual toxic shock syndrome (TSS), characterized by desquamation during a multisystem illness accompanied by fever. Recurrence of this effect, commonly within days and weeks of the first episode, has been known to happen and it appears to be traceable to the continued presence of *S. aureus* colonies on the skin. Lack of seroconversion after an acute episode of illness is a likely marker for those at risk for TSS recurrence (Andrews 2001).

Signs & Symptoms: Acute Gastrointestinal Exposure

SEB-induced illness via the gastrointestinal system normally manifests within a few hours of consuming the toxin (usually from infected foods). There is typically a sudden onset of nausea soon followed by vomiting, cramping abdominal pain, and diarrhea. The diarrhea is usually watery and not bloody. Anorexia is also common from this exposure. Dehydration may often arise in this syndrome. Fever can occur after oral exposure, though it is not very frequent (about 25% occurrence). The symptoms of gastrointestinal exposure tend to be quite incapacitating though recovery occurs within a few days or even several hours (Ulrich et al. 1997; IDEAS 2004).

Other signs of oral exposure may include tachycardia and hypotension. Hyperperistalsis and a diffuse non-localizing abdominal pain are frequently noted symptoms as well. (Williams 2004)

Connection to SIDS

In laboratory testing tissue insult by SEB in the intestines of rabbits included severe damage to the villi in the small intestine. Anesthetized rabbits exposed in this manner often suffered a trauma-free death from septic shock. This has led to the suggestion that SEB may play a role in sudden infant death syndrome (SIDS), especially after post-mortem examination of SIDS babies showed an association between intestinal tissue damage and fecal matter containing enterotoxins (Kamaras 2001a; Kamaras 2001b).

Signs & Symptoms: Acute Inhalation Exposure

After inhalation exposure, typically within 8-20 hours, a fever of up to 40 deg C is likely to commence, especially if significant pulmonary involvement has occurred. The fever may last 12 to 76 hours with associated chills. Myalgia is likely to occur as well. Headache is frequent, beginning in the first few hours after exposure and lasting for a few days, but growing noticeably milder on the second day (Ulrich et al. 1997).
Pulmonary Effects

Pulmonary effects typically include a non-productive cough manifesting 5-15 hours after intoxication with SEB and lasting 50-150 hours. Dyspnea and substernal chest pain are common as well. Rales are frequent, increasing with the severity of the illness and tend to be both inspiratory and expiratory. In severe cases rales are increasingly moist. Exertional dyspnea and rales can persist for several days (Ulrich et al. 1997).

Intense pleuritic substernal chest pain is often reported in the first or second day after exposure and can last for several days. Severe pulmonary compromise with profound dyspnea can occur in more severe intoxication; a patient may also present with adult respiratory distress syndrome (ARDS) (Ulrich et al. 1997).

Gastrointestinal Effects

Gastrointestinal signs after inhalation exposure include vomiting, nausea, and anorexia. Diarrhea is not usually part of the presentation of inhalation exposure to SEB, however. Anorexia can last as long as 140 hours. Vomiting is usually limited to one episode within the first day of intoxication, and can be the climax of an episode of coughing (Ulrich et al. 1997).

Other Symptoms

In one exposure case study, a patient recalled a burning eye sensation but no conjunctivitis was found (Ulrich et al. 1997).

Resolution/Outcome

Most of the above symptoms usually resolve in two weeks. A cough may endure for a full month (Williams 2004; Ulrich et al. 1997). Possible chronic effects of exposure are discussed in the following subsections on long-term effects.

Septic shock with a concomitant risk of death after acute inhalation exposure in high doses is also possible. Septic shock is caused by a drop in blood pressure induced by the toxin and the body’s response to it. Blood delivery to the organs becomes compromised and multiple organ failure may follow. Confusion and decreased consciousness are evident effects. Extremities turn bluish, pale and cool. A fever may be present but body temperature may alternatively or subsequently decrease. Rapid heartbeat, shallow rapid breathing, decreased urination and red patches on the skin are other symptoms of septic shock. Adult respiratory distress syndrome may also manifest (Anonymous 2004).

Death from SEB intoxication has been studied in rhesus monkeys. After 10 minutes of inhalation of a lethal amount of SEB, the monkeys first manifested gastrointestinal signs but then showed a long period of improvement. About 48 hours after exposure, however,
there was a 4-hour sudden and rapid decline, characterized by rapidly progressive lethargy, dyspnea, and facial pallor. Death followed (Ulrich et al. 1997).

**Acute Biologic Findings**

Biologic and laboratory signs of the effects of acute SEB respiratory exposure are not always evident or specific. Leukocytosis has been observed in patients several hours after SEB exposure. Hepatomegaly and bile in the urine have been reported, though both are exceptional. A normal chest X-ray may be found unless pulmonary involvement is severe. “Cuffing” (periobronchial accentuation) may be evident in moderate cases of SEB intoxication via inhalation. Pulmonary edema can occur, evidenced by densities on radiography. Kerley lines indicative of interstitial edema have been seen. Discoid atelectasis may be found on radiography during recovery (Ulrich et al. 1997).

An examination of rhesus monkeys exposed to lethal SEB concentrations found as the most striking pathologic feature a diffuse severe pulmonary edema. Edema fluid was found within the alveoli, alveolar septa, and the peribronchiolar, peribronchial, and perivascular interstitium. The precise pathology by which SEB caused the pulmonary lesions was not ascertained but the study’s authors suggested that cytokine production by activated T-cells might have induced vascular permeability changes which led to shock and highly marked pulmonary edema (Mattix et al. 1995).

Mildly elevated liver function tests have been seen in two cases of human exposure, one of whom had previous exposures to SEB from laboratory work (Ulrich et al. 1997).

Electrocardiograms are typically normal, even in the cases with chest pain, and blood pressure tends to remain stable. Elevated pulse rate tends to occur in tandem with changes in body temperature (Ulrich et al. 1997).

**Long-Term Effect: Possible Liver Function Test Elevation**

In a case report cited above, one of those afflicted manifested mildly elevated liver function tests which persisted for several years (unspecified exact duration in source). The patient also had multiple previous exposures to staphylococcal enterotoxins and the study authors were not convinced that the long-term elevated liver function test effect was a sequela of the exposure (Ulrich et al. 1997).

**Long-Term Effect: Allergy Diseases**

As a class, superantigen enterotoxins are increasingly being implicated in the development or course of many common diseases (Llewelyn 2002). For example, Kawasaki disease, a febrile vasculitis whose complications may turn out to be among the leading causes of acquired pediatric heart disease, and a significant cause of adult ischemic heart disease, is associated with exposure to superantigenic enterotoxins and their complex immune system activity (Cimaz 2003). Although SEB is not specifically
implicated, the role of enterotoxins in Kawasaki disease illustrates how superantigens may have much more serious long-term effects than the acute course of disease indicates (Leung 2002, Das 1996).

SEB has had significant observed associations with the development of atopic, ocular, and upper airway allergy diseases in individuals. SEB appears to be associated with specific allergic diseases like eczema (atopic eczema dermatitis syndrome – AEDS), asthma, psoriasis, and ocular conditions such as atopic conjunctivitis and vernal keratoconjunctivitis. Conclusive proof of causation remains elusive but associations are increasingly being established among SEB exposure, disease incidence, and the pathology of the conditions (Floret 2001).

**Atopic Dermatitis (AEDS)**

IgE antibodies to SEB have been positively correlated with more severe forms of adult atopic dermatitis. (Breuer 2000). It is also speculated that SEB may be a trigger factor in activating the inherited risk of AEDS. Evidence of exposure to SEB also has a demonstrated association with the exacerbation of atopic dermatitis in children (Sohn 2003). Recently, AEDS sufferers with high positive titers for SEA/SEB-specific IgE were shown to be more likely to have a more severe case of atopic dermatitis, more likely to be school children, more likely to have high serum concentrations of total IgE, more likely to exhibit exacerbation in summertime, and more likely to be pet owners of dogs and/or cats. (Ide 2004)

**Upper airway allergic disease**

Associations of SEB exposure with upper airway persistent allergic diseases have been reported (Heaton 2003). Recently, it was concluded that a status of sensitization to staphylococcal enterotoxins may have significance in the prognosis of such diseases. Specifically, staphylococcal enterotoxins SEA, SEB, SEC, and SED, along with toxin TSST-1, were found to be associated with higher serum eosinophil cationic protein (ECP), regarded as a strong marker of the severity of asthma and allergic rhinitis (Rossi 2004).

**Psoriasis Vulgaris**

An association of SEB with the chronic skin condition psoriasis vulgaris has also been advanced. A recent study tested the peripheral blood mononuclear cell (PBMC) response of patients with psoriasis vulgaris to staphylococcal superantigens SEA, SEB, and SEC1. Psoriasis vulgaris sufferers scored significantly higher than either normal subjects or patients with atopic dermatitis. When incubated with SEB, PMBCs secreted IL-2 and tumor necrosis factor (TNF-α) at an increased rate. The study authors concluded that it was possible that monocytes, in addition to T cells, are aggressively activated by staphylococcal superantigens so as to trigger or aggravate effects in psoriasis vulgaris patients, with the secreted cytokines acting as the mediators (Yamamoto 1998).
Chronic Ocular Effects: Atopic conjunctivitis and Vernal Keratoconjunctivitis

A recent examination of tears in patients with atopic conjunctivitis and vernal keratoconjunctivitis showed a positive correlation of those two conditions with SEB exposure. SEB antibodies were also higher in those more serious manifestations of the two diseases. The study authors concluded that SEB had a causative role in allergic diseases generally and that exposure to SEB may play an exacerbating role in the two conditions specifically (Shoji et al 2003).

Long-Term Effect: Autoimmune Diseases

Although as many as 3-5% of Americans suffer from autoimmune diseases, the etiology is complex and poorly understood. Most diseases are believed to involve both multigenetic and multiple environmental factors. Although no direct conclusive evidence of causation in humans has been shown, animal studies and biological plausibility both indicate that SEB and other superantigens may play a role in inducing a variety of autoimmune diseases. The autoimmune diseases that SEB exposure has been linked to include: Graves disease, arthritis, and multiple sclerosis (MS) (Llewelyn 2002).

Superantigens cause polyclonal activation of helper T-cells that can result in the breaking of self-tolerance to host antigens. Superantigens can also increase the production of TNF-α, which by itself, is known exacerbate many autoimmune diseases. Even if one can assume a causative relation exists, a key question that remains unanswered is whether a single exposure to SEB is sufficient to induce autoimmune diseases (Llewelyn 2002).

Graves disease

Graves disease is an autoimmune disease in which the immune system over-stimulates the thyroid gland resulting in the production of high levels of thyroid hormones. The upregulation of both HLA class I and class II molecules on thyroid cells are one of the main features of human thyroid autoimmunity. SEB has been shown, in the presence of lymphocytes, to upregulate the expression of both HLA class I and class II molecules on the surface of thyrocytes. Neutralizing antibodies to interferon-γ appeared to block upregulation of SEB-induced MHC molecules, indicating that upregulation is mediated by interferon-γ produced by SEB-stimulated T-cells. Glands taken from patients suffering for Graves disease had a more rapid upregulation of MHC molecules when treated with SEB than glands taken from non-immune subjects. Although these studies do not prove a causal association they provide biological plausibility for a role for superantigens in the etiology of Graves disease (Fierabracci et al. 1999).

Arthritis

Arthritis is a disease involving chronic inflammation of the joints. The two most common forms are rheumatoid arthritis and osteoarthritis, but over 100 different diseases
can present with joint inflammation. Both animal studies and observations from human clinical studies indicate that SEB can play a role in inducing or exacerbating arthritis. SEB induced a mild form of arthritis in female mice that were susceptible to collagen-induced arthritis. Surprisingly, no evidence of collagen-induced autoimmunity was actually seen in these mice (Omata et al. 1997).

SEB also caused severe arthritis in mice that had been previously immunized with bovine collagen (Nagai et al.1994; Nagai et al.1996a; Nagai et al.1996b). Treatment of arthritic mice with SEB has resulted in a demonstrable exacerbation of disease (Wooley et al. 1995). In humans, SEB has also been shown to induce the secretion of the chemokine PARC and the cytokine TNF-α; both of which have been associated with rheumatoid arthritis (Schutyser et al. 2001). IgM antibodies that reacted with SEB have been found in much higher concentration in patients with rheumatoid arthritis when compared to controls and Lupus patients (Origuchi et al. 1995).

**Multiple Sclerosis (MS)**

MS is an autoimmune disease causing inflammation of the central nervous system. Although the precise cause of MS remains elusive, activated T-cells that react with myelin basic protein (MBP) are believed to be responsible for much of the disease pathology. Animal studies have shown that SEB is able to reactivate paralysis in experimental allergic encephalomyelitis, the mouse model of MS. Superantigens induce epitope spreading, which results in the appearance of activated T-cells that react with previous subdominant epitopes of MBP (Soos et al. 2002). The majority of MBP reactive T-cell clones from MS patients are capable of being stimulated by superantigens. This indicates that superantigens such as SEB may play a role in either initiating or sustaining MS (Zhang et al. 1995).
V. PSYCHOGENIC EFFECTS

Psychogenic effects specific to SEB are not found in the literature. The general effects of perceived exposure to biological warfare agents are treated in the supplement “Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents.”
VI. TREATMENT & PREVENTION

Treatment for SEB exposure is generally limited to supportive care that varies depending on the severity of the illness. Careful attention should be paid to hydration in acute cases with vomiting and diarrhea; intravenous fluids may be necessary. Pulmonary challenge should be met with humidified oxygen, and steroids for pain control. If the exposure is severe, assisted ventilation and intubation with high oxygen concentrations may be called for. Steroids are not recommended for use in pulmonary edema and ARDS caused by SEB exposure. Otherwise, symptom-specific medications have proven useful in supportive treatment: acetaminophen for fever, codeine and dextromethorphan for cough, Compazine and Benadryl for nausea, and Darvon or codeine for headache pain (Ulrich et al. 1997; Williams 2004; IDEAS 2004; USAMRIID 2004).

Prevention against ordinary SEB exposure risk is proper storage of foodstuffs. In the case of risk of pulmonary exposure, which might happen in a biowarfare/bioterrorism context, protective masks are helpful (Ulrich et al. 1997, Williams 2004).

No vaccine is officially approved for use against SEB. Nevertheless, an inactivated recombinant staphylococcal enterotoxin B vaccine has been in development at the U.S. Army’s Medical Research Research Institute of Infectious Diseases. The vaccine is based on a mutation of key amino acid residues in the major histocompatibility complex (MHC) class II binding sites of SEB that prevents the MHC binding but retains antigenic properties that allow the immune system to respond to wild-type SEB. Tests on mice and rhesus monkeys have been promising (Boles 2003).

Pirfenidone, an anti-fibrotic compound currently under development by Intermune to treat pulmonary fibrosis and other cytokine mediated illnesses, was shown to be effective in reducing or eliminating the effects of SEB in mice. The effect was time dependent with protection seen when pirfenidone was administered 2-4.5 hours after exposure. Pirfenidone also inhibited in a dose-dependent fashion the release of cytokines from SEB-stimulated PBMC cells. The protective effect of pirfenidone is believed to be primarily due to its inhibition of TNF-α synthesis and release. TNF-α release is believed to be responsible for many of the toxic effects of SEB, particularly cytokine-mediated acute respiratory distress syndrome and inflammatory lung disease, following inhalation. (Hale et al. 2002). Other compounds, such as niacinamide and pentoxifylline, which also inhibit TNF-α production have also been shown to reduce the toxicity of SEB (Krakauer et al. 1999).

An affinity-purified chicken anti-SEB antibody has shown some promise for use as a prophylactic measure. In rhesus monkeys challenged with a lethal aerosol concentration (135µg/kg), monkeys given the chicken antibody both before and after four hours of SEB administration survived, though with signs of intoxication challenge (Ulrich et al. 1997).
Removing SEB contamination from an area is usually but performed by a 10-15 minute washing with a solution of sodium hypochlorite, which can be made from a ratio of 9 parts water to 1 part household bleach (USAMRIID 2004; IDEAS 2004).
VII. SECONDARY SOURCE COMMENTARY

Secondary sources concerned with the potential of biological weapons use of SEB, including specifically those addressing health effects of Project SHAD agents (e.g. “Project 112 Glossary”), tend to overlook the possible associations of SEB with chronic allergy related disorders, SIDS, and autoimmune diseases. (DeploymentLink 2004; Ulrich et al. 1997; USAMRIID 2004; IDEAS 2004; Williams 2004) This may be because the associations or mechanisms are tenuously established, and do not reflect the prevailing concerns over the acute traumatic potential of biowarfare.
VIII. BIBLIOGRAPHY WITH ABSTRACTS

Special note

Unless otherwise stated, abstracts accompanying the following references are rendered verbatim as provided by the original publication or as provided from a standard print or electronic catalogue or database. Errors, omissions, or other defects of style or substance are strictly those of the original source.

The susceptibility of Staphylococcus aureus strains isolated from human clinical and non-clinical sources in Trinidad to bacteriophages and antimicrobial agents was determined. The ability of the strains to produce enterotoxins and toxic shock syndrome toxin-1 (TSST-1) was also investigated. Of the 554 strains tested, 454 (81.8%) were susceptible to international phage set (IPS) phages with strains isolated from bacteruria (57.1%) and bacteremia (53.3%) having a low sensitivity compared to isolates from aspirates (87.3%) and anterior nares (97.4%). All sources combined, strains were most susceptible to phages belonging to several groups (mixed). Overall, 419 (75.6%) strains were resistant to one or more of nine antimicrobial agents tested. Resistance to penicillin was most prevalent, with 413 (74.5%) strains found to be resistant. Prevalence of resistance to tetracycline, gentamicin, oxacillin, cefuroxime and ciprofloxacin was 5.1%, 2.0%, 0.7%, 0.4% and 0.4%, respectively. Of the 554 strains tested, 307 (55.4%) produced staphylococcal enterotoxins A (SEA), B (SEB), C (SEC) and D (SED) singly or in combination. Strains recovered from high vaginal swabs were least enterotoxigenic (40.0%) as compared to umbilical infection isolates which were most enterotoxigenic (78.9%). TSST-1 was produced by 95 (19.0%) out of 499 strains tested, with isolates from bacteruria found to be most toxigenic (33.3%). It was concluded that the S. aureus strains tested were highly susceptible to bacteriophages and antimicrobial agents (except penicillin) and that enterotoxigenic and TSST-1 producers were widespread and have an aetiologic potential.

We report 3 cases of recurrent nonmenstrual toxic shock syndrome (TSS) and review the clinical manifestations, diagnosis, and treatment. The primary sites of infection were the genital tract (in a patient who underwent cesarean delivery), the upper respiratory tract, and a breast abscess. In all 3 patients, the initial illness was not recognized to be TSS; only after development of recurrent illness with desquamation was this diagnosis entertained. Strains of Staphylococcus aureus that were isolated from 2 patients produced
TSS toxin-1, whereas the third strain produced staphylococcal enterotoxin B. All 3 patients lacked antibody to the implicated toxins at the time of presentation with recurrent illness. Nonmenstrual TSS can occur in a variety of clinical settings and may be recurrent. The presence of desquamation during a febrile, multisystem illness could suggest this diagnosis and should prompt the clinician to obtain appropriate cultures for S. aureus.


Toxin-mediated diseases have made humans ill for millennia. They also have been used in beneficial ways. Unfortunately, the use of biological agents as weapons of terror has now been realized, and separating naturally occurring disease from bioterroristic events has become an important public health goal. The key to timely identification of such attacks relies on education of primary care physicians, first responders, and public health officials. We must remain vigilant to unusual case presentations or clusters of similar cases and report them immediately to public health authorities.


At this time there are no vaccines or therapeutics to protect against staphylococcal enterotoxin B (SEB) exposure. Here, we report vaccine efficacy of an attenuated SEB in a nonhuman primate model following lethal aerosol challenge and identify several biomarkers of protective immunity. Initial in vitro results indicated that the mutation of key amino acid residues in the major histocompatibility complex (MHC) class II binding sites of SEB produced a nontoxic form of SEB, which had little to no detectable binding to MHC class II molecules, and lacked T-cell stimulatory activities. When examined in a mouse model, we found that the attenuated SEB retained antigenic structures and elicited protective immune responses against wild-type SEB challenge. Subsequently, a vaccine regimen against SEB in a nonhuman primate model was partially optimized, and investigations of immune biomarkers as indicators of protection were performed. SEB-naive rhesus monkeys were vaccinated two or three times with 5 or 20 microg of the attenuated SEB and challenged by aerosol with wild-type SEB toxin. Unlike exposure to the native toxin, the vaccine did not trigger the release of inflammatory cytokines (TNF alpha, IL6, or IFN gamma). All rhesus monkeys that developed anti-SEB serum titers > or = 10(4) and elicited high levels of neutralizing antibody survived the aerosol challenge. These findings suggest that the attenuated SEB is fully protective against aerosolized toxin when administered to unprimed subjects. Moreover, experiments presented in this study identified various biomarkers that showed substantial promise as correlates of immunity and surrogate endpoints for assessing in vivo biological responses in primates, and possibly in humans, to vaccines against SEs.

Sigma receptors originally described in distinct regions of the central nervous system are expressed on cells of the immune system. A sigma ligand, SR 31747A, was observed here to inhibit in vitro the Staphylococcal enterotoxin B (SEB)-driven lymphocyte proliferation. In mice, the drug confers a potent protection against the lethality induced by SEB, stimulates the SEB-induced serum release of interleukin (IL)-10 and inhibits at the same time the systemic release of IL-2, IL-4, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6 and tumour necrosis factor-alpha (TNF-alpha). The enhancement of IL-10 production by this compound is also effective in nude mice treated with SEB, indicating that IL-10 of T-cell origin is not involved in this process. The finding that a sigma ligand protects against the SEB-induced toxicity provides insights into the clinical use of this family of compounds, particularly in food poisoning and septic shock where Staphylococcal enterotoxins are involved. The observation that this compound stimulates IL-10 synthesis indicates that it could be a potent regulatory agent of chronic inflammatory diseases.


**BACKGROUND:** Staphylococcus aureus has been identified as a possible trigger factor in atopic dermatitis (AD). Some 30-60% of S. aureus strains isolated from patients with AD are able to produce exotoxins with superantigenic properties, mostly staphylococcal enterotoxins A, B, C, and D (SEA-D) and toxic shock syndrome toxin-1 (TSST-1). Recently, it was demonstrated that the presence of IgE antibodies to SEA and SEB is correlated with the severity of skin lesions in children with AD. To determine the relevance of staphylococcal enterotoxins in adult patients with AD, we investigated the relationship between the severity of skin lesions and sensitization to SEA and SEB.

**METHODS:** Clinical severity was determined by the SCORAD index. Circulating IgE antibodies to SEA and SEB, serum eosinophil cationic protein (ECP) levels, and urine eosinophil protein X (EPX) levels were measured. **RESULTS:** The skin condition was significantly worse in patients sensitized to SEB than in unsensitized patients. Serum ECP and urine EPX levels were found to be significantly higher in SEB-sensitized patients, confirming the higher degree of cutaneous inflammation. **CONCLUSIONS:** Our results demonstrate a relationship between severity of skin lesions and sensitization to SEB in adult patients with AD, but a relationship between disease activity and sensitization to SEA could not be shown.

**Castle, et al. 1999.** Age-related impaired proliferation of peripheral blood mononuclear cells is associated with an increase in both IL-10 and IL-12. *Exp.Gerontol.* 34(2): 243-252.

Reflective of age-associated decline in immune function among elderly individuals is a decrease in in vitro T cell proliferative ability. Impaired T cell proliferation in the elderly may result from disruption of the well-balanced network of regulatory cytokines produced during an immune response. The purpose of this study was to identify age-
related changes in the production of interleukin (IL)-10 and IL-12, and to determine whether in vitro T cell proliferation can be enhanced in the elderly by modulation of these two key cytokines. The superantigen Staphylococcus enterotoxin B (SEB) was used to stimulate proliferation and IL-10 and IL-12 production in peripheral blood mononuclear cells (PBMC) in vitro. Proliferation was determined by standard tritiated thymidine uptake. Cytokine levels in culture supernatants were measured by ELISA. We observed impaired SEB-induced proliferation of PBMC in the elderly that is comparable to that seen with the polyclonal mitogen Con A. This age-related decline in proliferation was associated with increased production of both IL-10 and IL-12. Modulation of PBMC proliferative response with either recombinant IL-12 or IL-10-neutralizing antibodies can boost proliferation of elderly PBMC to the levels seen in unmodulated young controls.


While it is well established that cellular activation can increase human immunodeficiency virus (HIV) replication in T lymphocytes, it is also clear that both activated CD8+ and CD4+ T lymphocytes mediate anti-HIV activity. To assess the relative importance of these contrary effects on HIV replication in vivo, we evaluated the consequences of Mycobacterium bovis BCG and staphylococcal enterotoxin B (SEB) inoculation in vivo in rhesus monkeys chronically infected with simian immunodeficiency virus of macaques (SIVmac). BCG inoculation induced as much as a 2.5-log reduction of plasma and intracellular SIV RNA in SIVmac-infected monkeys. This down-regulation of virus replication persisted as long as 4 weeks after BCG inoculation. Similarly, SEB injection resulted in up to a 3-log decrease in plasma and intracellular SIV RNA in SIVmac-infected macaques. Interestingly, the short-term reduction of viremia in these monkeys correlated with the peak in vivo production of SEB- and BCG-induced cytokine responses. However, no long-term clinical benefit was observed in the SIVmac-infected macaques. These studies provide in vivo evidence that potent T-cell stimulation driven by antigens other than the virus itself can, under some circumstances, mediate short-term reduction of viremia in AIDS virus-infected individuals.


Kawasaki disease (KD) is a febrile systemic vasculitis complicated by coronary and peripheral arterial aneurysms in 20-35% of untreated patients. It is reported as the commonest cause of acquired heart disease in children in developed countries, and may be a risk for adult ischaemic heart disease. Although KD has been reported all over the world, it is overexpressed among Asian populations, especially Japanese. The disease pathogenesis is still unknown and several theories have been proposed, including the possibility of an infection by a toxin-secreting microorganism and of a superantigen-driven process. Despite numerous efforts there is still no diagnostic test available for KD, and the diagnosis is based on clinical criteria after the exclusion of other diseases.
presenting with high persistent fever. Prompt diagnosis is critical, since the early administration of intravenous immunoglobulins and aspirin reduces the rate of coronary abnormalities to less than 5% of patients.


Experimental allergic encephalomyelitis (EAE) is inducible in experimental animals immunized with myelin basic protein (MBP), proteolipid protein (PLP) or their peptides. We compared T-cell responses to encephalitogenic epitopes of PLP(43-64) and MBP(Ac1-11) in a single mouse strain, (PL/J x SJL)F1. MBP(1-11)-specific T-cell hybridomas expressed predominantly TCR V beta 8 or V beta 4, while PLP(43-64)-specific hybridomas expressed a diverse TCR repertoire. To analyze the biologic significance of the TCR repertoire (limited vs. diverse) to disease susceptibility, we pretreated mice with a superantigen (SEB), and then induced disease with these autoantigens. Mice injected with SEB and immunized with MBP(Ac1-11) showed significant inhibition of EAE, whereas SEB-pretreated mice immunized with PLP(43-64) had an increased severity of EAE and developed a chronic disease. These data demonstrate that prior exposure to microbial superantigens can significantly alter the autoimmune disease course depending upon the TCR repertoire used by the autoantigen.


Cholera toxin (Ctx) from *Vibrio cholerae* and the closely related *Escherichia coli* heat-labile enterotoxin (EtX) are the primary virulence factors responsible for causing cholera and traveller's diarrhea, respectively. Studies on the mode of action of these toxins on gut epithelial cells have revealed important insights into the mechanisms of toxin uptake and trafficking in eukaryotic cells. However, of perhaps even greater fascination have been the discoveries that Ctx and EtX exhibit remarkable immunological properties. When either of these toxins is administered via mucosal routes, it triggers a potent mucosal and systemic anti-toxin immune response. By contrast, local or systemic immunization with other soluble protein antigens usually stimulates only a meagre immune response, or results in a state of immunological tolerance. Even more striking are the findings that when Ctx or EtX are mixed with heterologous antigens, they function as adjuvants, leading to stimulation of mucosal responses to the admixed antigen, and the abrogation of oral tolerance. In addition, recent observations have shown that the receptor-binding component of these toxins can down-regulate inflammatory diseases associated with the induction of autoimmune disorders such as rheumatoid arthritis, diabetes, and multiple sclerosis. While the underlying mechanisms responsible for these remarkable properties have yet to be resolved, it is clear that the toxins' ability to bind to cell surface receptors plays an important role in their potent immunogenicity, adjuvanticity, and immunotherapeutic properties. This review provides an overview of the latest
developments within the Ctx/Etx field, with a special emphasis on the cell entry
mechanisms and immunomodulatory action of Ctx/Etx and their component subunits.

Oral delivery represents one of the most pursued approaches for large-scale human
vaccination. Due to the different characteristics of mucosal immune response, as
compared with systemic response, oral immunization requires particular methods of
antigen preparation and selective strategies of adjuvanticity. In this paper, we describe the
preparation and use of genetically detoxified bacterial toxins as mucosal adjuvants and
envisage the possibility of their future exploitation for human oral vaccines.

**DeploymentLink. 2004.** *Project 112 Glossary.* Deployment Health Support,
Undersecretary of Defense (Personnel and Readiness).
http://deploymentlink.osd.mil/current_issues/shad/shad_glossary.shtml

**Di Marco, et al. 1998.** Deoxyspergualin neither counteracts lipopolysaccharide (LPS) or
Staphylococcus aureus enterotoxin-B (SEB) induced lethality in mice nor does it
To gain further insights into the immunopharmacological mode of action of the
immunosuppressant antibiotic deoxyspergualin (DSP), its effects were evaluated in
murine lethal endo- and exotoxemia. These are two cytokine-mediated macrophage and T
cell dependent immunoinflammatory conditions that can be induced in D-Galactosamine
(D-Gal) presensitized mice by the injections with either LPS or SEB, respectively. The
results show that prophylactic treatment with DSP (2.5 or 5 mg/kg bd.wt. 48, 24 and 2 h
prior to challenge) neither improved the rate of survival, nor influenced the massive
increase in the blood levels of tumor necrosis factor-alpha which followed the challenge
with LPS or SEB. In sharp contrast, these clinical and seroimmunological events were
both markedly counteracted by prophylactic treatment with sodium fusidate, another
immunosuppressive agent used as control.

34, table of contents.
This article reviews the literature regarding the structure and function of two types of
exotoxins expressed by Staphylococcus aureus, pyrogenic toxin superantigens (PTSAgs)
and hemolysins. The molecular basis of PTSAg toxicity is presented in the context of two
diseases known to be caused by these exotoxins: toxic shock syndrome and
staphylococcal food poisoning. The family of staphylococcal PTSAgs presently includes
toxic shock syndrome toxin-1 (TSST-1) and most of the staphylococcal enterotoxins
(SEs) (SEA, SEB, SEC, SED, SEE, SEG, and SEH). As the name implies, the PTSAgs
are multifunctional proteins that invariably exhibit lethal activity, pyrogenicity,
superantigenicity, and the capacity to induce lethal hypersensitivity to endotoxin. Other
properties exhibited by one or more staphylococcal PTSAgs include emetic activity (SEs)
and penetration across mucosal barriers (TSST-1). A detailed review of the molecular
mechanisms underlying the toxicity of the staphylococcal hemolysins is also presented.
The etiology of almost half of the diarrheal diseases has not been cleared yet, in spite of modern diagnostic methods. Bacteroides fragilis strains which secrete an enterotoxin are termed as enterotoxigenic B.fragilis (ETBF). These strains are associated with diarrheal diseases in children above 1 year of age and in adults. B. fragilis toxin (BFT) stimulates intestinal secretion and in-vitro cytotoxic response in HT29/C1 cells. Recent studies suggest that BFT is related to inflammatory bowel disease and colon cancer by triggering nuclear activation with potential oncogene expression. In this review, the molecular pathogenesis, epidemiology and laboratory diagnosis of ETBF have been reviewed to focus on ETBF as a diarrheal agent.

The proinflammatory cytokine, tumour necrosis factor alpha (TNFalpha) has been shown to play a pivotal part in mediating acute and chronic inflammation. The activities of TNFalpha are modulated by the proteolytic shedding of the soluble extracellular domains of the two TNF receptors, p55 sTNF-RI and p75 sTNF-RII. Amgen Inc has cloned and expressed a recombinant form of a natural inhibitor of TNFalpha, referred to as recombinant human soluble TNF receptor type I (r-Hu-sTNF-RI, sTNF-RI). sTNF-RI is an E coli recombinant, monomeric form of the soluble TNF-type I receptor. A high molecular weight polyethylene glycol (PEG) molecule is attached at the N-terminus position to form the molecule intended for clinical evaluations (PEG sTNF-RI). Preclinical studies to date demonstrate that PEG sTNF-RI is efficacious in rodent models of chronic inflammatory disease including rheumatoid arthritis and Crohn's disease at doses as low as 0.3 mg/kg given every other day. This dose results in plasma concentrations of 0.3 to 0.5 microg/ml. Higher doses with correspondingly higher plasma concentrations yield higher efficacy. It has also demonstrated efficacy in E coli lipopolysaccharide, and Staphylococcus enterotoxin B mediated models of acute inflammation in rodents and primates. Pharmacokinetic studies in mice, rats, cynomolgus monkeys, baboons, and chimpanzees have been conducted with PEG sTNF-RI. Absorption from a subcutaneous dose was slow, with the time to reach maximal plasma concentrations of 24-48 hours in rats, and in monkeys, and 3-29 hours in chimpanzees. The initial volume of distribution of PEG sTNF-RI was essentially equivalent to that of plasma (40 ml/kg). This suggests the protein does not appear to extensively distribute from the systemic circulation with a volume of distribution at steady state (Vss) less than 200 ml/kg in all species studied. These results are consistent with previous experience with PEGylated proteins in which PEGylation decreases both the rate of absorption and the plasma clearance of human recombinant proteins in animals and humans. The use of a PEG molecule will probably provide a more advantageous dosing schedule (that is, less frequent dosing) for the patient compared with a non-PEG sTNF-RI.

Successful application of the next generation of vaccines will require that protection be induced with a minimal number of administrations, and that a practical approach to inducing immunity at mucosal surfaces be developed. For these reasons, vaccine-containing microspheres were formulated from the biodegradable and biocompatible copolymer poly(DL-lactide-co-glycolide) [DL-PLG]. Subcutaneous immunization of mice with 1- to 10-microns microspheres containing a toxoid vaccine of staphylococcal enterotoxin B (SEB) induced a 500-fold potentiation of the circulating antitoxin response. Strong adjuvant activity was dependent on the microspheres being no more than 10 microns in diameter and required that the antigen was within the particles. The rate of DL-PLG biodegradation is a function of the ratio of lactide to glycolide, and the coinjection of SEB toxoid microspheres formulated with two different DL-PLG ratios stimulated both a primary and an anamnestic secondary antitoxin response. When it was administered by the oral or intratracheal (IT) route, microencapsulated SEB toxoid was found to be effective in the induction of concurrent circulating and disseminated mucosal antibody responses. Female rhesus macaques immunized with a microencapsulated simian immunodeficiency virus (SIV) vaccine produced high levels of circulating anti-SIV antibodies, and following oral or IT boosting, specific antibodies were found in vaginal wash fluids. Vaginal challenge with viable homologous SIV resulted in the infection of three out of four nonimmunized but only one out of seven microsphere-immunized macaques. Thus, DL-PLG microspheres are a promising approach to the delivery of vaccines, combining adjuvant activity with controlled release and effective presentation to mucosally associated lymphoid tissues (MALT).


Enterotoxin was detected in 22 (61.1%) of the 36 S. aureus strains isolated from clinical materials and in 3 (13%) of the 23 S. aureus strains from food samples (P < 0.05). On the basis of individual types of enterotoxin, staphylococcal enterotoxin A (SEA) was produced by 11.1%, SEB by 38.9% and SEC by 22.2% of SS. aureus strains from clinical material. Of the food S. aureus strains, SEC and SED produced by 8.7% and 4.3% respectively. Of the clinical and food S. aureus strains, 52.8% and 39.1%, respectively, were typeable by the 23 phages of International Phage Set. The majority of the typeable S. aureus strains from clinical and food sources belonged to group II being at 22.2% and 17.4% respectively. Furthermore, of the 14 SEB-producing S. aureus, 42.9% were of phage group II. In conclusion, the results obtained indicate that enterotoxin-producing S. aureus strains from clinical materials in Libya are not uncommon; however, certain foods appear not to be the source of such strains. Because of the low susceptibility to bacteriophages shown by S. aureus isolated in Libya, compared to reports from several countries, other methods of typing should be used in conjunction with phage typing in epidemiological investigations concerning this organism.

Eugster, et al. 2001. Superantigen overcomes resistance of IL-6-deficient mice towards MOG-induced EAE by a TNFR1 controlled pathway. Eur. J. Immunol. 31(8): 2302-2312. Experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein peptide 35-55 (MOG) leads to a chronic form of disease characterized by demyelination, inflammation and gliosis in the central nervous system (CNS). Recently IL-6 and LT alpha were found to be required for induction of the disease. The main features associated with EAE resistance of IL-6(-/-) and LT alpha(-/-) mice were reduced T cell proliferation and endothelial activation. As shown here treatment of MOG-immunized IL-6(-/-) mice with staphylococcal enterotoxin B (SEB) reversed their resistance to MOG-induced EAE. SEB failed to restore susceptibility to EAE in LT alpha(-/-) mice. The effect of SEB to induce EAE in IL-6(-/-) mice depends on TNF receptor type 1 (TNFR1) signaling because IL-6/TNF/LT alpha(-/-) and IL-6/TNFR1(-/-) are refractory to SEB. TNFR1 is involved in SEB induced trafficking of T cells into the CNS as evidenced by the failure to up-regulate VCAM-1 on CNS endothelium and lack of accumulation of V beta 8(+) T cells in the CNS of IL-6/TNFR1(-/-) mice upon immunization with MOG and treatment with SEB. The course of SEB triggered EAE in MOG immunized IL-6(-/-) mice was characterized by reduced severity and duration of clinical manifestations, which were associated with a significant drop of CNS infiltrating neutrophils and MIP-2 expression after peak disease. Taken collectively the effect of SEB to overcome EAE resistance points to a transient IL-6 independent but TNFR1 dependent proinflammatory pathway in EAE pathogenesis and suggests a crucial function for IL-6 in disease perpetuation.


"Superantigens" is the term for a group of molecules that have in common an extremely potent stimulatory activity for T lymphocytes of several species. They stimulate CD4+, CD8+ and gamma delta + T cells by a unique mechanism: they cross-link variable parts of the T-cell receptor (TCR) with MHC class II molecules on accessory or target cells. The interaction site on the class II molecule and on the TCR is different from the peptide binding site; on the TCR it is the variable part of the beta chain (V beta). The prototype superantigen is the staphylococcal enterotoxin B (SEB), member of a family of genetically related proteins produced by Staphylococcus aureus and Streptococcus pyogenes. These are soluble exotoxins of approximately 27 kd molecular mass. It is intriguing that this molecular mechanism of T-cell stimulation has been independently produced at least three times in evolution. Other pathogens producing superantigens are retroviruses (the Mouse Mammary Tumor Viruses) and a mycoplasma (Mycoplasma arthritidis). Many additional candidate superantigens have been proposed, but in most cases unequivocal evidence for superantigen activity is still missing. There are several reasons why these molecules have aroused such tremendous interest in recent years. First, they have provided key information on tolerance mechanisms, both on the deletion of T cells in the thymus and on the induction of peripheral tolerance by anergy and apoptosis. Second, of all polyclonal T-cell stimulators they are the ones that most closely mimic the recognition of specific antigen. Finally, they have been recognized as important factors in the pathogenicity of the producing pathogens, inducing shock and immunosuppression.
Whilst there is evidence that superantigens could be involved in the pathogenesis of certain human diseases, in most cases this is still very preliminary and indirect.


Microbial superantigens have been implicated in the pathogenesis of human autoimmune diseases. In autoimmune glands, thyrocytes inappropriately express HLA-DR molecules and these cells may function as antigen presenting cells (APC) We studied the effect in vitro of staphylococcal enterotoxin B (SEB) on HLA molecule expression on thyrocytes obtained from autoimmune and non-autoimmune glands by immunofluorescence. HLA class I and class II upregulation could be detected by FACS analysis on thyrocytes. Anti-IFN-gamma neutralizing antibodies markedly affect both class I and class II upregulation on thyrocytes. FRTL5 cells were not responsive to SEB. Similarly, a human thyroid cell strain maintained in culture in a conditioned medium was not induced to express HLA products by SEB stimulation. The addition of autologous intrathyroidal lymphocytes caused reestablishment of the SEB.


Staphylococcus aureus and Streptococcus pyogenes produce a lot of toxins, some of them responsible for specific diseases. Staphylococcal food poisoning is due to ingestion of enterotoxin containing food. Seven toxins have been isolated so far. Generalized exfoliative syndrome is related to exfoliatin. Young children are particularly affected. The disease consists in a cutaneous exfoliation usually limited with a favourable outcome. The mucus membranes are not involved. The nose or pharynx are the most usual portal of entry. Staphylococcus aureus is not grown from the bullae. Severe extensive forms have been observed particularly in neonates (Ritter's disease). Bullous impetigo is also due to exfoliatin. It consists in the presence of a restricted number of cloudy bullae, from which staphylococcus can be grown. It is a mild disease with a favourable outcome within a few days. Scarlet fever is related to the streptococcal erythrogenic toxins. The classic form of the disease is presently rare. This disease may be related to staphylococcus as a complication of arthritis, osteomyelitis or wound superinfection. Bacteremia is usual. Staphylococcal scarlet fever is not related to exfoliatin as previously believed, but to enterotoxins or TSST-1, so it seems to be an abortive form of toxic shock syndrome. Toxic shock syndrome is defined as a multi organ failure syndrome with a rapid onset, fever, rash followed by desquamation, vomiting and diarrhea, hypotension, conjunctivitis and strawberry tongue. The disease is related to an infection or colonisation with a toxin (TSST-1) producing strain of Staphylococcus aureus. Enterotoxins (mainly C) may be involved. The disease may occur in childhood, sometimes after superinfection of varicella. The mortality is low (5%) and mainly due to ARDS or cardiac problems. Erythrogenic toxins produced by Streptococcus pyogenes are involved in a streptococcal form of toxic shock syndrome with a quite similar presentation. In most cases however, a cutaneous or soft tissue infection is at the origin. Necrotizing fasciitis complicating varicella is a classic cause in children. Bacteremia is
often observed. The mortality rate is as high as 60%. The streptococcal strains involved in north America use to produce the toxin erythrogenic A, the european cases seem to be more related to strains secreting the B toxin with a dysregulation of the mechanisms which control the secretion of the toxin. Staphylococcus strains producing the Panton and Valentine leucocidin are responsible for chronic or relapsing furunculosis and above all for a very severe necrotizing pneumonia observed in children and young adults presenting as an acute respiratory distress syndrome with leucopenia, hemoptysis and shock carrying a heavy mortality rate. Besides these specific diseases, staphylococcal and streptococcal toxins may be involved in some syndromes of unknown origin, in which the intervention of superantigens seems very likely. Kawasaki syndrome is among them as strains producing staphylococcal and streptococcal toxins have been grown from patients with Kawasaki syndrome. In the same way, the intervention of toxins is suspected in the determination of sudden infant death syndrome and atopic eczema.


Induction of mucosal immunity by oral immunization with protein antigen alone is difficult: potent mucosal adjuvants, vectors, or other special delivery systems are required. Cholera toxin (CT) has been shown to be an effective adjuvant for the development of mucosal vaccines and, when given with vaccine, induces both mucosal and systemic immune responses via a Th2 cell-dependent pathway. However, and in addition to potential type-I hypersensitivity, a major concern for use of mucosal adjuvants such as CT is that this molecule is not suitable for use in humans because of its inherent toxicity. When we examined the potential toxicity of CT for the central nervous system, both CT and CT-B accumulated in the olfactory nerves/epithelium and olfactory bulbs of mice when given by the nasal route. The development of effective mucosal vaccines for the elderly is also an important issue; however, only limited information is available. When mucosal adjuvanticity of CT was evaluated in aged mice, an early immune dysregulation was evident in the mucosal immune system. The present review discusses these potential problems for effective mucosal vaccine development. Tolerance represents the most common and important response of the host to environmental antigens, including food and commensal bacterial components, for the maintenance of an appropriate immunological homeostasis. We have examined whether Peyer patches could play a more important role for the maintenance of oral tolerance. Using Peyer patch-null mice, we found that mice lacking this gut-associated lymphoid tissue retained their capability to produce secretory IgA antibodies but did not develop normal oral tolerance to protein antigens.


To study the mechanisms underlying the development of interstitial pneumonia in autoimmune disease, we analyzed bronchoalveolar lavage fluid (BALF) in an animal model of interstitial pneumonia in which an intratracheal instillation of staphylococcal enterotoxin B (SEB) induced interstitial pneumonia in autoimmune-prone mice. Increases
in the numbers of total cells, macrophages, lymphocytes, and neutrophils were observed in BALF from SEB-treated MRL +/+ mice, and peaked at 3 d after SEB administration (Day 3). Flow cytometric analyses revealed increases in SEB-reactive Vbeta8(+) T cells, indicating that SEB-reactive cells play an important role in bronchoalveolar space. The expressions of tumor necrosis factor (TNF)-alpha, interferon (IFN)-gamma, JE/monocyte chemoattractant protein-1, regulated on activation, normal T cells expressed and secreted, and KC/gro messenger RNA (mRNA) in BALF cells from SEB-treated mice peaked at Day 3. Increased expression of TNF-alpha mRNA was observed mainly in macrophages and CD8(+) T cells, and the increase in IFN-gamma mRNA was observed mainly in CD8(+) T cells in BALF at Day 3. The expression of platelet-derived growth factor mRNA was very weak at Day 3 but strongly expressed at Day 14. An immunosuppressant, FK506, but not corticosteroid, suppressed SEB-induced T-cell expansion in BALF as well as increased cytokine and chemokine production in the bronchoalveolar space of SEB-treated mice. Histologically, FK506 but not corticosteroid significantly reduced both the cell infiltration to alveolar septal walls and the synthesis of pulmonary collagen fibers. Further, transfer of T cells of MRL +/+ mice with SEB into SCID mice gave rise to interstitial pneumonia. These results suggest that superantigen-reactive T cells in the bronchoalveolar space may trigger the development of interstitial pneumonia in this model.


A case of a 73-year-old woman with acute renal failure due to toxic shock syndrome (TSS) is reported. The patient was admitted to our hospital with the complaints of high fever, disturbance of consciousness and shock. Laboratory findings on admission were: CRP 25.11 mg/dl, WBC 35000/ microl, Plt 1.6 x 10(4)/ microl, GOT 155 U/l, GPT 65 U/l, CPK 4202 U/l (CPK-MM 96%), BUN 123 mg/dl and SCr 7.0 mg/dl. Because of anuria, hemodialysis was performed. This patient was treated with dopamine, methyl prednisolone (MP), frozen fresh plasma, AT III, antibiotics, and platelet transfusion. The bacterial cultures of blood and cerebrospinal fluid were negative, but MRSA was isolated subsequently from the pharynx and vagina. We investigated the production of toxic shock syndrome toxin 1 (TSST-1) and staphylococcal enterotoxins (SE). The isolated MRSA produced TSST-1, SEB and SEC. Accordingly, we made the diagnosis of TSS. After improvement of acute renal failure and the patient's general condition, MRSA persisted and TSST-1 was still found in the patient's blood. Finally we eradicated the MRSA and TSST-1 after administration of ciprofloxacin hydrochloride (CPFX) and Rifampicin (RFP).


We developed PCR-enzyme linked immunosorbbent (ELISA) assays to detect Staphylococcus aureus enterotoxins A and B genes. The assays use internal biotin-labelled oligonucleotides as capture probes for immobilizing and subsequently detecting
target sequences on microtiter plates. The detection limits of the PCR-ELISAs were approximately 250 gene copies, versus 2500 gene copies by agarose gel analysis. The sensitivity of the assays, as determined from a reference panel of 46 coded samples that included DNA purified from 31 different bacterial species and strains, SEA and SEB plasmid controls, and no-template controls was 100%. No cross-reactivity was observed with DNA from non-staphylococcal species. Using 27 clinical isolates of S. aureus, the SEA PCR-ELISA identified the enterotoxin A (sea) gene in 26 samples, and the SEB PCR-ELISA identified the enterotoxin B (seb) gene in all 27 samples. Compared with conventional antigen capture ELISAs for SEA and SEB toxins, the PCR-ELISAs showed overall superior detection limits. The sensitivity and specificity levels of the SEA PCR-ELISA and the SEA toxin ELISA were comparable within their respective detection thresholds, but the sensitivity and specificity of the SEB PCR-ELISA was much greater than that of SEB toxin ELISA.


Repeated ligation of the TCR results in apoptosis (activation-induced cell death; AICD). Superantigens such as Staphylococcal enterotoxin B (SEB) are particularly efficient at inducing AICD in T cells. We investigated whether apoptosis in human T cell subsets was due to fratricide (killing of neighboring cells) or suicide (cell autonomous death). AICD of Th1, Th2, Tc1, and Tc2 effector cells was dramatically enhanced at low cell densities and could be observed in single cell microcultures. AICD was unaffected by adhesion molecules or neighboring cells undergoing AICD, confirming the predominance of a suicidal mechanism. However, SEB was able to induce fratricidal apoptosis of type 1, but not type 2 cells. Fratricide was also observed when unstimulated T cells were exposed to activated Tc1 effector cells. Thus, AICD is tightly regulated to allow clonal T cell expansion and memory cell generation, but superantigens may subvert this process by allowing T cell fratricide.


Microbiological, biological, and chemical toxins have been employed in warfare and in terrorist attacks. In this era, it is imperative that health care providers are familiar with illnesses caused by these agents. Botulinum toxin produces a descending flaccid paralysis. Staphylococcal enterotoxin B produces a syndrome of fever, nausea, and diarrhea and may produce a pulmonary syndrome if aerosolized. Clostridium perfringens epsilon-toxin could possibly be aerosolized to produce acute pulmonary edema. Ricin intoxication can manifest as gastrointestinal hemorrhage after ingestion, severe muscle necrosis after intramuscular injection, and acute pulmonary disease after inhalation. Nerve agents inhibit acetylcholinesterase and thus produce symptoms of increased cholinergic activity. Ammonia, chlorine, vinyl chloride, phosgene, sulfur dioxide, and nitrogen dioxide, tear gas, and zinc chloride primarily injure the upper respiratory tract and the lungs. Sulfur mustard (and nitrogen mustard) are vesicant and alkylating agents. Cyanide poisoning ranges from sudden-onset headache and drowsiness to severe
hypoxemia, cardiovascular collapse, and death. Health care providers should be familiar with the medical consequences of toxin exposure, and understand the pathophysiology and management of resulting illness.


Pirfenidone [5-methyl-1-phenyl-2-(1H)-pyridone] down-regulates expression of cytokines and other mediators involved in the onset and development of pulmonary fibrosis. Pirfenidone also inhibits production of tumor necrosis factor alpha (TNF-alpha) from macrophages incubated with endotoxin and protects mice against endotoxin shock. Pirfenidone’s ability to reduce cytokine expression in these disorders led us to investigate the drug’s effect on another cytokine anomaly, superantigen-induced shock. BALB/c mice were exposed to staphylococcal enterotoxin B (SEB) either systemically or by aerosol and subsequently potentiated with a sublethal dose of lipopolysaccharide. In these experiments, pirfenidone given 2 to 4.25 h after SEB resulted in 80 to 100% survival versus only 0 to 10% survival among untreated control animals. Relative to serum cytokine levels from controls given toxin but no drug, there was a 35 to 80% decrease in TNF-alpha, interleukin 1, and other proinflammatory cytokines. In vitro experiments with human peripheral blood lymphocytes revealed that pirfenidone reduced SEB-induced cytokine levels 50 to 80% and inhibited 95% of SEB-induced T-cell proliferation.

Overall, these studies demonstrated the potential utility of pirfenidone as a therapeutic against septic shock and the biological effects of SEB.


Staphylococcus aureus produces a set of proteins (e.g., staphylococcal enterotoxin A [SEA], SEB, toxic shock syndrome toxin 1 [TSST-1]) which act both as superantigens (SAgs) and toxins. Although their mode of action as SAgs is well understood, little is known about how they enter the body via the intestine and cause food poisoning. To examine this problem we used an in vitro culture system to study the capacity of class II MHC-negative human intestinal epithelial cells (Caco-2) to transcytose several staphylococcal toxins. We found that Caco-2 cells are capable of dose-dependent, facilitated transcytosis of SEB and TSST-1, but not SEA. We extended these studies in vivo in mice by showing that ingested SEB appears in the blood more efficiently than SEA. Our data suggest that these toxins can cross the epithelium in an immunologically intact form. These results may have important implications for the pathogenesis of food poisoning.


Both experimental and clinical forms of chronic GVHD have unique immunological features. The affected animals/individuals suffer from autoimmune disorders such as systemic lupus erythematosus (SLE), and yet they are unable to mount a self MHC-restricted T cell response to foreign antigens. Pathogenesis of the latter phenomenon was
investigated in an experimental model of chronic GVHD. Chronic GVHD was induced in 8-10-week-old (B6xC3H)F1 mice by tail vein injection of 5 x 10(7) spleen cells of C3H parental strain. The recipients, when tested 3 months later, were unable to mount a T helper (Th) cell response to a randomly selected immunogen, a vaccine of 10(8) killed Mycobacterium vaccae. The animals showed evidence of generalized lymphoid hyperplasia, as indicated by GVH index >1.34, and also revealed autoantibodies against erythrocytes and dsDNA, indicating establishment of chronic GVHD. However, mice with chronic GVHD of only 3 weeks duration were able to mount the Th cell response to M. vaccae. Three consecutive immunizations of these mice at 1-week intervals, with the same immunogen, resulted in the mice becoming non-responsive to the antigen. All the three responses tested, namely the DTH, lymphoproliferation and the antibody responses, were adversely affected. The non-responsiveness induced was antigen-specific. Mice receiving two immunizations with M. vaccae responded normally to Salmonella enteritidis. Pulse treatment with cyclosporin A 0.5 mg/mouse by the i.p. route, on days 0, 1, 2, 3 and 4 at the time of immunization with M. vaccae on day 1, prevented emergence of non-responsiveness. Based on this evidence, it was concluded that repeated activation of T cells of mice with chronic GVHD induces non-responsiveness. Extent of clonal loss due to activation-induced cell death (AICD) caused by i.p. injection with a superantigen Staphylococcal enterotoxin B (SEB) was investigated in F1 mice with chronic GVHD. I.p. injection of 25 microg/mouse of SEB induced loss of SEB responding clones in both normal F1 mice and those having chronic GVHD; however, the extent of loss was much greater in the latter. In vitro antigen-specific proliferation of primed splenic T cells of normal F1 mice was observed to be quite poor when antigen was presented by APC of mice with chronic GVHD of 3 weeks duration. Proliferation profiles of T cells of normal F1 mice, in response to stimulation with concanavalin A (Con A) or SEB, were studied, using as APC irradiated spleen cells of normal F1 mice or of F1 mice with chronic GVHD of 3 weeks duration. With Con A and APC of normal F1 mice, peak proliferation was observed at 48 h, which remained at the same level up to 72 h and declined thereafter, possibly due to AICD. With SEB and the normal APC, proliferation progressively peaked at 72 h and declined thereafter. With APC of mice with chronic GVHD, the 48 h proliferative responses of both Con A and SEB were comparable to those caused by APC of normal F1 mice; however, thereafter the responses declined steeply, suggesting greater AICD. Based on these results, it was concluded that APC of mice with chronic GVHD are functionally altered to induce greater AICD.


This study examined the emetic activity of several staphylococcal enterotoxin type A and B (SEA and SEB, respectively) mutants that had either one or two amino acid residue substitutions. New sea gene mutations were constructed by site-directed mutagenesis; gene products were obtained with glycine residues at position 25, 47, 48, 81, 85, or 86 of mature SEA. Culture supernatants from Staphylococcus aureus RN4220, or derivatives containing either sea or a sea mutation, were analyzed for the ability to stimulate proliferation of murine splenocytes, as determined by incorporation of [3H]thymidine. Culture supernatants containing SEA-N25G (a SEA mutant with a substitution of glycine
for the asparagine residue at position 25), SEA-F47G, or SEA-L48G did not stimulate T-cell proliferation, unlike supernatants containing the other substitution mutants. Purified preparations of SEA-N25G had weak activity and those of SEA-F47G and SEA-L48G had essentially no activity in the T-cell proliferation assay. All mutants except SEA-V85G, which was degraded by monkey stomach lavage fluid in vitro, were tested for emetic activity. SEA-C106A and two SEB mutants, SEB-D9N/N23D and SEB-F44S (previously referred to as BR-257 and BR-358, respectively), whose construction and altered immunological properties have been reported previously, were also tested in the emetic assay. Each mutant was initially administered intragastrically at doses of 75 to 100 micrograms per animal; if none of the animals responded, the dose was increased four-to-fivefold. SEA-F47G, SEA-C106A, and SEB-D9N/N23D were the only mutants that did not induce vomiting at either dose tested; these three mutants had reduced immunological activity. However, there was not a perfect correlation between immunological and emetic activities; SEA-L48G and SEB-F44S retained emetic activity, although they had essentially no T-cell-stimulatory activity. These studies suggest that these two activities can be dissociated.


G-CSF has immunomodulatory effects of neutrophilic granulocytes and monocytes/macrophages. Two studies were done: one in normal volunteers and the other in HIV-infected patients plus their respective control donors to evaluate the effect of Filgrastim on cytokine responses. Filgrastim treatment of volunteers resulted in an anti-inflammatory cytokine response, when blood was stimulated ex vivo with the endotoxin lipopolysaccharide (LPS). Similarly, in the presence of Filgrastim in vitro, the LPS-inducible release of proinflammatory cytokines was attenuated. Blood from HIV-infected patients at advanced stages of disease showed reduced interleukin (IL)-2 formation in response to staphylococcal exotoxin B (SEB), which was restored in the presence of Filgrastim.


BACKGROUND: The incidence of Staphylococcus aureus (S. aureus) colonization on the skin of patients with atopic eczema/dermatitis syndrome (AEDS) is approximately 90% and a variety of evidence implicates epidermal staphylococcal infection as a pathogenic factor in atopic dermatitis. However, the mechanism(s) underlying the effects of this organism in the disease process are unclear. The cellular responses of AEDS suffers and asymptomatic atopic individuals to bacterial superantigens (SAg) were investigated in an attempt to elucidate the role of staphylococcal enterotoxin B (SEB) in atopic disease. METHODS: Peripheral blood mononuclear cells (PBMC) were isolated from normal nonatopic adults, asymptomatic atopic individuals, patients with active AEDS and patients with active allergic asthma. The cells were cultured for 24 or 96 h with house dust mite (HDM), SEB and phytohaemaglutinin (PHA), and the supernatants were assayed for cytokine levels. RESULTS: Staphylococcal enterotoxin B selectively stimulates the production of interleukin (IL)-5 in AEDS sufferers but not in...
asymptomatic atopics or nonatopics. Additionally, we observed comparable susceptibility to the IL-5-stimulatory effects of SEB in allergic asthmatics. CONCLUSIONS: Given the central role of IL-5-driven eosinophilia in progression from mild atopy to severe disease, these findings provide a plausible mechanism for the AEDS-promoting effects of staphylococcal SAg. Staphylococcal enterotoxin B may also have a similar role in atopic respiratory disease.


The bacterial superantigen staphylococcal enterotoxin B (SEB) induces in vivo a state of anergy defined by the inability of V beta 8+ CD4+ T cells to produce IL-2 upon restimulation in vitro. However, restimulation in vivo triggers a burst of acutely released lymphokines including IL-2 and TNF, paralleled by up-regulation of lymphokine-specific mRNA. Since anergy as defined in vitro appears not to operate in vivo, we analyzed parameters able to induce responsiveness in anergic T cells. We show here that in vitro stimulation of anergic T cells with competent Ag-presenting cells induces responsiveness, provided the APC (activated B cells or dendritic cells) present high concentrations of SEB. Crosslinking of CD28 molecules on anergic T cells could substitute the requirement for competent APC. Quantitation of TCR threshold by determining the SEB concentrations able to trigger half-maximal T cell responses revealed that anergic and normal T cells exhibited the same TCR threshold for the expression of functional IL-2 receptors (IL-2R), yet the TCR threshold for induction of IL-2 production was 10- to 100-fold elevated in anergic T cells. TCR threshold for normal and anergic T cells was further dependent on the type of APC, i.e., costimulus-competent APC required 100-fold less SEB. The results indicate that extrinsic factors such as ligand concentration and costimulus competence of APC can overcome the heightened TCR threshold of anergic T cells, thus reverting anergy into responsiveness.


This preliminary study investigated the potential role of staphylococcal superantigens in the pathogenesis of canine pyoderma. The staphylococcal enterotoxins A (SEA), SEB, SEC and SED, and the toxic shock syndrome toxin-1 (TSST-1) were assayed in isolates from skins of dogs with pyoderma. Culture supernatants from 25 of 96 isolates were positive for multiple superantigens, with SEA and SEC being the most frequently detected. In in vitro stimulation of canine peripheral blood mononuclear cells and quantitative flow cytometry revealed that low concentrations of SEA and SEB were potent stimulators of blastogenesis of T cells.

Superantigens combine with MHC class-II molecules to form the ligands that stimulate T cells via the V beta element of the T-cell receptor. Two groups of superantigens have been described so far: first, endogenous murine products that include the Mls determinants, and second, bacterial products such as the Staphylococcal enterotoxins. Here, we review studies that address the interactions between the foreign superantigens and MHC class-II molecules, the mechanism of T-cell stimulation, and the role that tolerance to self-superantigens plays in shaping the T-cell repertoire. We speculate on the possible evolutionary significance of superantigens.


The most widely used in vitro measure of T-cell function has been the assessment of mitogen induced proliferation by [(3)H]-thymidine incorporation. Mitogens also induce T-cell surface expression of a number of molecules associated with activation, including CD69. Recent reports have suggested that flow cytometric analysis of CD69 expression may be a simpler and faster means of measuring T-cell function. Most studies have been on normal subjects, and the sensitivity of CD69 expression as an in vitro measure of clinical immunodeficiency remains unknown. We address this issue by concurrently measuring mitogen-stimulated T-cell CD69 expression and [(3)H]-thymidine incorporation in a normal population and five immunocompromised patients negative for the human immunodeficiency virus (HIV). All patients had recurrent infections and had known causes of immunodeficiency. Whole blood cultures were setup to measure phytohaemagglutinin A (PHA-) and superantigen staphylococcal enterotoxin B (SEB)-induced CD69 expression at 5, 24, and 72 h, and [(3)H]-thymidine incorporation at 72 h. All immunodeficient patients had lower than normal PHA responses and 3 of 4 had low SEB responses. However in 7 out of 8 of the patient tests, mitogen-induced T-cell CD69 expression was within the normal range. Similar results were found with CD4(+) T-cell CD69 expression. This study indicates that measurement of mitogen-induced T-cell CD69 expression lacks sensitivity in determining T-cell dysfunction in HIV-negative immunodeficient patients.


*Background:* The authors clarified the clinical significance of the measurement of serum concentrations of specific IgE antibodies to staphylococcal enterotoxin (SE) A- and SEB in atopic dermatitis (AD).

*Methods:* The serum concentrations of SEA- and SEB-specific IgE antibodies in 140 pediatric patients with AD were measured with an immuno CAP radioallergosorbent test system (RAST). To check the cross-reaction of specific IgE antibodies to SEA/SEB and other allergens, the CAP RAST fluorescent enzyme immunoassay inhibition test was performed.

*Results:* Forty-seven patients (33.6%) tested positive for either SEA- or SEB-specific IgE antibodies. School children showed higher positive rates of SEA/SEB-specific IgE antibodies than infants or young children. The patients with severe AD and those with exacerbation of symptoms in summer, had higher positive rates of SEA/SEB-specific IgE antibodies than patients with mild AD or those with exacerbation in winter. In addition,
the positive rates of specific IgE antibodies to both dog-dander and cat-dander were higher in patients with positive SEA/SEB-specific IgE antibodies than in patients with negative ones. No cross-reactions occurred among specific IgE antibodies to SEA/SEB and dog/cat dander with one patient's serum, which had positive IgE-specific antibodies against cat/dog dander and SEA/SEB. The positive rate of SEA/SEB-specific IgE antibodies in the patients with dogs and/or cats as pets was 48.4%, which was higher than in those with no pets. 

**Conclusions:** Atopic dermatitis patients who exhibit high positive rates of SEA/SEB-specific IgE antibodies were found to be school children, severe cases, cases with high serum concentrations of total IgE, cases with exacerbation in summer, and cases with dogs and/or cats as pets. The measurement of serum concentrations of specific IgE antibodies to SEA and SEB, thus has some value for evaluating AD patients.


**BACKGROUND:** Mycophenolate mofetil (MMF), an ester prodrug of mycophenolic acid (MPA), is a potent immunosuppressive agent used in clinical organ transplantation. MPA preferentially inhibits the type II isoform of inosine monophosphate dehydrogenase, depletes GTP, suppresses transfer of mannose and fucose to glycoproteins, and prevents lymphocyte proliferation in vivo. Whether MMF can also delete activated T cells in vivo by triggering an apoptotic signal was addressed in this study. To this end we analyzed the activity of MMF in mice injected with the bacterial superantigen staphylococcal enterotoxin B (SEB). Superantigens bind to MHC class II molecules without requirement for processing, and activate subsets of CD4+ and CD8+ T cells whose T cell receptor beta chains express Vbeta family-specific homologous sequences. This model that shares several features with direct allorecognition has the unique advantage of allowing a precise monitoring of activated T cells. **METHODS:** BALB/c mice treated with MMF (100 mg/kg/day) or vehicle were injected with SEB. Serum cytokines, CD4+ and CD8+ Vbeta8+ cells were monitored in blood and lymphoid tissues, and apoptosis was determined by externalization of membrane phosphatidyl serine, double strand DNA breaks, and expression of B220 antigen by Vbeta8+ cells. **RESULTS:** MMF treatment decreased tumor necrosis factor alpha, interferon gamma, and interleukin-10 secretion induced by SEB. It did not modify other early activation events (blast transformation, CD69 and CD25 expression) but completely inhibited SEB-induced expansion of Vbeta8+ cells by inducing apoptosis of SEB-reactive T cells. A similar effect was observed in CD95-ligand-deficient mice. Repeated SEB injections associated with MMF resulted in a marked decrease of CD8+ Vbeta8+ T cells. SEB-induced increase of Vbeta8+ thymocytes was not prevented by MMF treatment. **CONCLUSION:** Results obtained in this in vivo model suggest that MMF treatment may induce deletion of activated peripheral T cells and decrease early cytokine responses.
OBJECTIVE: To explore the effects of apoptosis of peripheral blood lymphocytes (PBLs) of patients with chronic hepatitis B (CHB) on the persistent infection of hepatitis B virus (HBV). METHODS: The PBLs of 15 patients with CHB were isolated and cultured with or without Staphylococcus aureus enterotoxin B (SEB; 0.2 mg/L), or in the presence or absence of recombinant HBeAg (rHBeAg; 1.0 mg/L) for 48 hours in vitro. After incubations, the cells were harvested by centrifugation and then apoptosis of the PBLs was studied by staining with fluorescent dyes YOPRO-1 and Hoechst33342. RESULTS: The percentage of apoptotic cells of PBLs of patients with CHB was significantly higher than in normal controls (P < 0.01) in the presence or absence of SEB or rHBeAg, but the ratio of apoptotic cells in rHBeAg-stimulated PBLs of the patients was the highest, reaching (24.6 +/- 6.1)% The patients with seropositive for HBeAg had higher percentage of apoptotic cells in their cultured PBLs than those with seronegative for HBeAg had, but the ratio of apoptotic cells in cultured PBLs of the patients with chronic heavy and severe hepatitis B was lower than that of the patients with chronic mild and moderate hepatitis B (P < 0.01). CONCLUSION: Activation-induced cell death of PBLs of the patients with CHB may be related to persistent infection of HBV, and assay of apoptosis of PBLs of patients with CHB may provide valuable information about pathogenesis of CHB.


The aim of this project was to characterise the type of damage caused to the intestine of the infant rabbit by bacterial enterotoxins implicated in sudden infant death syndrome (SIDS). Samples of the duodenum, jejunum, ileum, caecum and large intestine exposed to the toxins for up to 6 hours were examined by scanning (SEM) and transmission electron microscopy (TEM). The damage was quantitatively assessed (% villi damaged) by SEM and qualitatively by SEM and TEM. Clostridium perfringens enterotoxin, staphylococcal enterotoxin B and Clostridium difficile toxin A + toxin B combined all caused severe damage to the villi in the small intestine (80-90% damage). Clostridium difficile toxin B caused only slight damage (17% to the jejunum, 26% to the caecum). Clostridium perfringens alpha-toxin caused moderate damage to the small intestine (duodenum 34%, caecum 35%), and Escherichia coli STa caused significant damage to the small (53-70%) and large intestine (51%). The level of toxin damage increased with time, the small intestine being more susceptible generally to damage than the large intestine. Each toxin differed in its ability to damage the villi, microvilli, enterocytes and lamina propria.


Sections of the duodenum, jejunum, ileum, caecum and large intestine from 14 sudden infant death syndrome (SIDS) babies were examined by scanning (SEM) and transmission electron microscopy (TEM). The type and amount of damage was characterised and quantitated and compared with the presence of Clostridium perfringens,
Clostridium difficile, Escherichia coli and Staphylococcus aureus in faecal samples from the babies and toxins from the bacteria in faecal samples and serum from the babies. The data were compared with the damage that these toxins cause to the rabbit intestinal epithelium (see the previous paper in this issue). Damage was present in most of the SIDS samples, varying from 0 to 96%, and most damage occurred when the faecal samples contained the above bacteria and their toxins. Damage varied from removal of microvilli, damage to villus tips, separation of and removal of epithelial cells from the lamina propria, and removal of enterocytes leaving goblet and tuft cells, to damage and breakdown of the lamina propria. The results support the hypothesis that the cause of death in a significant proportion of SIDS babies may result from the absorption of toxins from the intestinal tract initiating a toxic shock reaction.


Enterohemorrhagic Escherichia coli (EHEC) are the most common cause of postdiarrheal hemolytic-uremic syndrome (HUS). The most important EHEC serotype implicated worldwide is O157:H7. However, several so-called non-O157 EHEC serotypes have emerged. After a mean incubation period of 3 days, patients develop watery diarrhea accompanied by cramping abdominal pain. During the next days, in most patients watery diarrhea changes to bloody diarrhea. One week after the onset of diarrhea, in about 15% of infected patients under 10 years of age EHEC infection results in a systemic complication, HUS. Shiga toxins (Stxs) are considered the major virulence factors of EHEC involved in the pathogenesis of HUS. It is generally believed that after intestinal infection with EHEC, Stxs cross the intestinal barrier and bind to endothelial cells. At this point they presumably injure the host cell by inhibition of protein synthesis, stimulation of prothrombotic messages, or induction of apoptosis. The B subunit of Stx binds to the membrane receptor globotriaosylceramide (Gb3). Gb3 facilitates the endocytosis and intracellular trafficking of the toxin. The Stx A subunit hydrolyzes a specific adenine residue of the 60S ribosomal subunit of mammalian cells. As a consequence, Stx shuts down the protein machinery of the susceptible cell. The HUS is the net effect of a variety of interacting factors, including background risk of acquisition, host factors (such as age), virulence characteristics of the infecting EHEC strain, and exogenous factors. All known EHEC virulence determinants are located on mobile genetic elements, and this has an important impact on the evolution of these pathogens. The evolution of EHEC has a dynamic component that includes different genetic mechanisms. The recent progress in understanding the pathogenesis and epidemiology of EHEC infections forms a basis for the development of future strategies to prevent EHEC infections in humans.


Sensitive, rapid and reproducible detection of staphylococcal enterotoxin B (SEB) in a range of different biological matrices was achieved using the ORIGEN((R)) Immunoassay System (Igen, Inc). The homologous immunoassay format consisted of a
double antibody sandwich in which a biotinylated capture antibody, pre-bound to streptavidin-coated paramagnetic beads, was used to bind antigen from test samples. A detector antibody, labeled with ruthenium (II) tris-bipyridal chelate, was added and, when bound to the bead immunocomplex, generated light in the presence of an excess of tripropylamine. The light was detected and measured by the ORIGEN analyzer. The sensitivity of this assay was 1 pg of enterotoxin per ml of serum, urine, tissue, or buffer and was highly reproducible. Concentration curves generated from SEB standards produced consistently wide linear ranges (0.1-100 ng/ml), making quantitation possible with only two dilutions of sample (undiluted and 1:1000). The assay used 50 microl of sample per test and required a 30 min incubation period in addition to a 1 min per tube reading time (50 tubes maximum). This assay was significantly better in terms of sensitivity, linear range, and assay time than the standard microplate enzyme-linked immunosorbent assay and should permit early SEB detection in clinical samples, food, and environmental samples.


Microbial superantigens (SAg), including SEB and TSST-1, polyclonally activate T cells belonging to specific TCR BV families. A pathogenic role for SAg in various human diseases has been suggested, but enthusiasm for this view has been tempered by the T cell oligoclonality in these disorders. To assess whether T cell oligoclonality can emerge following protracted SAg stimulation, human PBMC were stimulated with SEB, TSST-1, or anti-CD3 mAb and maintained in culture with exogenous IL-2. Oligoclonality was appreciated by day 14 among CD4(+) and CD8(+) T cells. In addition, mice transgenic for human DR2 and DQ8 were injected weekly with SEB, and splenic CD4(+) and CD8(+) T cells were analyzed for oligoclonality. In mice that received one or three such injections, little-to-no oligoclonality was detected. In contrast, considerable oligoclonality was detected in mice that received eight weekly SEB injections. Many of these T cell oligoclonal clones were identical to "spontaneously" arising oligoclonal clones detected in SEB-naive mice. Thus, T cell oligoclonality can emerge following chronic SAg stimulation. In hosts who have lost tolerance to self Ag, chronic exposure to SAg may preferentially promote expansion of autoreactive T cells and facilitate development of clinical disease.


Tumor necrosis factor alpha (TNF-alpha) is a critical cytokine that mediates the toxic effects of bacterial superantigens like staphylococcal enterotoxin B (SEB) and toxic shock syndrome toxin 1 (TSST-1). Pentoxifylline, an anti-inflammatory agent that inhibits endotoxemia and lipopolysaccharide (LPS)-induced release of TNF-alpha, was tested for its ability to inhibit SEB- and TSST-1-induced activation of human peripheral blood mononuclear cells (PBMCs) in vitro and toxin-mediated shock in mice. Stimulation of PBMCs by SEB or TSST-1 was effectively blocked by pentoxifylline (10 mM), as evidenced by the inhibition of TNF-alpha, interleukin 1beta (IL-1beta), gamma interferon (IFN-gamma), and T-cell proliferation. The levels of TNF-alpha, IL-1alpha,
and IFN-gamma in serum after an SEB or TSST-1 injection were significantly lower in mice given pentoxifylline (5.5 mg/animal) versus control mice. Additionally, pentoxifylline diminished the lethal effects and temperature fluctuations elicited by SEB and TSST-1. Thus, in addition to treating endotoxemias, the cumulative in vitro and in vivo data suggest that pentoxifylline may also be useful in abrogating the ill effects of staphylococcal enterotoxins and TSST-1.


Since the early work of Mischell & Dutton (Science 153, 1004-1008, 1966), it has been recognized that certain lymphocyte cultures are exquisitely sensitive to the harsh effects of atmospheric oxygen tension. The influence of oxygen partial pressure (pO2) on normal human peripheral blood mononuclear cell (PBMC) phenotype, proliferative ability, cytokine, immunoglobulin production, and redox status was examined by culturing PBMC under ambient oxygen (high pO2) or a more physiological pO2 (5% O2; low pO2). Low pO2 conditions promoted a significant increase in overall viable PBMC number and enhanced Concanavalin A (Con A) - or pokeweed mitogen (PWM)-stimulated PBMC proliferation by approximately 30% and 50%, respectively. No differential pO2 effects were apparent on phytohemagglutinin (PHA) - or staphylococcal enterotoxin B (SEB)-induced proliferation. Both resting and Con A-stimulated lymphocytes incubated for 24 h under high pO2 had a greater baseline carboxy-2',7'-dichlorofluorescin (C-DCF) fluorescence, and were less able to quench the effect of H2O2 treatment compared to lymphocytes cultured under low pO2 conditions. Supernatant gamma-IFN, IL-2, and IL-4 concentrations were elevated 50-65% when PBMC were stimulated with Con A for 24 h under low pO2; however, lipopolysaccharide (LPS)-stimulated IL-1 beta production was reduced by over 75%. PWM-stimulated IgM production by PBMC was significantly reduced in day 7 cultures incubated under low pO2, whereas IgG and IgA production remained relatively unaltered. Immunophenotyping analyses did not reveal any significant alterations in cell subset or marker distribution at the time points examined; however, an interesting trend of increased CD69 expression was observed for Con A-stimulated PBMC incubated under low pO2. These results demonstrate that O2 is a critical parameter for the in vitro culture of lymphocytes, and suggests that varying pO2 may differentially alter PBMC functionality.


*Clostridium difficile* is a spore forming, gram-positive anaerobic bacillus first described in 1935 by Hall and O'Toole as a commensal organism in the fecal flora of healthy newborn infants (1). The organism was given its unusual name because it grew slowly and was difficult to isolate in pure culture. Its presence in the stool of healthy neonates suggested that *C. difficile* was a nonpathogen, even though it produced toxins in broth culture. Following its original description, *C. difficile* passed quickly into relative
obscurity in the 1960's and 1970's when antibiotic-associated pseudomembranous colitis became prevalent following the introduction into clinical practice of broad spectrum antibiotics. The frequent association of clindamycin and lincomycin therapy with pseudomembranous colitis led to the term “clindamycin colitis” (2). A breakthrough occurred in 1978 when C. difficile was identified as the source of a cytotoxin in the stool of patients with pseudomembranous colitis (3). During the two decades since its rediscovery, a great deal has been learned about the pathophysiology, epidemiology and management of C. difficile infection, yet many challenges remain. Currently this organism infects over 30% of individuals admitted to United States hospitals, making C. difficile colitis one of the most common nosocomial infections (4). It is estimated that approximately 10-12 million adults are infected with this organism each year in the United States, about a third of whom become symptomatic. The disease burden in the elderly is particularly severe as they are hospitalized more frequently and for longer duration. The pathophysiology of C. difficile diarrhea requires alteration of the colonic microflora by antibiotics, colonization by C. difficile, and release of two potent enterotoxins designated A and B (5). The toxins of Clostridium difficile are required virulence factors in both animals and humans since non-toxigenic strains do not cause disease. Recent cloning and sequencing of the toxin genes reveals extensive amino acid homology between them that is reflected in common molecular and cellular mechanisms. Both toxins damage cells by modifying the rho family of proteins, key regulators of cellular actin. C. difficile infection causes a florid acute inflammatory response seen in patients with pseudomembranous colitis. It is now realized that neurons and immune cells of the lamina propria are major determinants of toxin-induced diarrhea and mucosal damage. Early critical events following toxin exposure are release of the neuropeptides substance P and calcitonin gene related peptide (CGRP) from sensory afferent neurons and activation of lamina propria macrophages and intestinal mast cells. These peptides in turn release a complex cascade of other inflammatory mediators from lamina propria cells (5). The importance of the host immune response, specifically serum IgG directed against toxin A, is now recognized as a critical determinant of disease expression in man.


Staphylococcal enterotoxins (SE) interact with major histocompatibility complex (MHC) class II cell-surface receptors, eliciting signal transduction in antigen-presenting cells (APC). Subsequent toxin-class II complex interaction with specific T-cell receptors induces T-cell activation. We investigated the effect of niacinamide and interleukin (IL)-10 on SEB-induced responses. In a macrophage cell line, niacinamide (ED50--2mM) and IL-10 (ED50--7U/ml) inhibited interferon (IFN)-gamma-induced MHC class II expression in a dose-dependent manner. Also, niacinamide was a potent inhibitor of T-cell proliferation induced by SEB (ED50-- 1 mM) while IL-10 has minimal effects. In mice, the temporal responses of IL-1alpha, tumor necrosis factor (TNF)-alpha, IL-2, and IFN-gamma evoked by SEB were synergistically potentiated by lipopolysaccharide (LPS). Lethality occurred only when SEB was potentiated by LPS. Niacinamide or IL-10 improved survival of mice after lethal SEB challenge. Niacinamide reduced cytokine serum levels, although the pattern differed from that of IL-10. Niacinamide primarily
reduced IL-2 and IFN-gamma, while IL-10 predominantly reduced IL-1alpha and TNF-alpha. The immunomodulatory effects of niacinamide observed on SEB-induced activation of APC and T-cells in vitro and in the LPS potentiated murine model for SEB-induced toxicity suggest it may have therapeutic value.

Bioterrorism is an emerging public health and infection control threat. Potential biological agents include smallpox, anthrax, plague, tularemia, botulinum toxin, brucellosis, Q fever, viral encephalitis, hemorrhagic fever, and staphylococcal enterotoxin B. An understanding of the epidemiology, clinical manifestations, and management of the more likely candidate agents is critical to limiting morbidity and mortality from a biological event. Effective response requires an increased index of suspicion for unusual diseases or syndromes, with prompt reporting to health authorities to facilitate recognition of an outbreak and subsequent intervention. Hospital epidemiology programs will play a crucial role in this effort.


OBJECTIVES: To assess the prevalence of superantigen secreting bacteria in children with acute Kawasaki disease (KD) relative to control patients. STUDY DESIGN: Bacterial cultures were obtained in a blinded fashion from the throat, rectum, and groin of 45 patients with untreated acute KD and 37 febrile control patients from 6 centers in the United States. Cultures were processed for the presence of superantigen-producing bacteria at a central laboratory. RESULTS: Staphylococci or streptococci that produced superantigens (TSST-1, SEB, SEC, SPEB, SPEC) were isolated from 25 of 45 patients with KD (56%) as compared with 13 of 37 (35%) control patients (P =.078). Because SEB- and SEC-producing Staphylococcus aureus have not been associated with KD and because they do not induce a Vbeta2+ T-lymphocyte response, we analyzed the difference between groups relative to superantigens TSST-1 or SPEB/SPEC production. TSST-1 secreting S aureus or SPEB/SPEC producing group A streptococci were isolated from 20 of 45 (44%) patients with KD compared with 7 of 37 (19%) control patients (P =.019). CONCLUSIONS: The overall isolation rates of superantigen (TSST-1, SPEB, SPEC, SEB, SEC) producing bacteria between patients with KD and febrile control patients were not statistically significant. However, future studies should further examine the potential role of Vbeta2-stimulatory superantigens (TSST-1 and SPEB/SPEC) in KD.

The array biosensor is capable of detecting multiple targets rapidly and simultaneously on the surface of a single waveguide. Sandwich and competitive fluoroimmunoassays have
been developed to detect high and low molecular weight toxins, respectively, in complex samples. Recognition molecules (usually antibodies) were first immobilized in specific locations on the waveguide and the resultant patterned array was used to interrogate up to 12 different samples for the presence of multiple different analytes. Upon binding of a fluorescent analyte or fluorescent immunocomplex, the pattern of fluorescent spots was detected using a CCD camera. Automated image analysis was used to determine a mean fluorescence value for each assay spot and to subtract the local background signal. The location of the spot and its mean fluorescence value were used to determine the toxin identity and concentration. Toxins were measured in clinical fluids, environmental samples and foods, with minimal sample preparation. Results are shown for rapid analyses of staphylococcal enterotoxin B, ricin, cholera toxin, botulinum toxoids, trinitrotoluene, and the mycotoxin fumonisín. Toxins were detected at levels as low as 0.5 ng mL\(^{-1}\).

**Lindberg, et al. 2000.** Long-time persistence of superantigen-producing Staphylococcus aureus strains in the intestinal microflora of healthy infants. *Pediatr.Res.* 48(6): 741-747. Staphylococcus aureus has been isolated at an increasing rate from infants' stools during the last decades, but it is not known whether this species can colonize and persist in the intestinal microflora. To investigate this, 49 Swedish infants were followed prospectively from birth until 12 months of age. S. aureus was identified in a rectal swab obtained 3 d after delivery and in quantitative cultures of fecal samples collected at 1, 2, 4, and 8 weeks and at 6 and 12 months of age. A random amplified polymorphic DNA (RAPD) method was developed to distinguish individual S. aureus strains from one another and the strains were tested for production of enterotoxins A-D and TSST-1. By 3 days of age, 16% of infants had S. aureus in their intestines, which increased to 73% by 2-6 months, whereafter it decreased slightly to 53%. At the same time S. aureus population counts in colonized infants declined from an average 10\(^{6.8}\) CFU/g feces during the first months of life to 10\(^{4.0}\) CFU/g feces by 12 months. Colonized infants usually harbored one or two S. aureus strains in their microflora for long periods of time. Few strains were transient passengers and the median time of persistence of S. aureus strains in the microflora was several months. Of the 75 S. aureus strains identified, 43% produced one or more toxins: 13% SEA, 7% SEB, 23% SEC, 4% SED, and 11% TSST-1. Altogether, 47% of the investigated infants were colonized by a toxin-producing S. aureus during their first year of life. Despite this they were apparently healthy and did not have more gastrointestinal problems than noncolonized infants. This report is the first to show that S. aureus may be a resident member of the normal intestinal microflora in infancy.

**Lingwood. 1999.** Glycolipid receptors for verotoxin and Helicobacter pylori: role in pathology. *Biochim.Biophys.Acta.* 1455(2-3): 375-386. Eukaryotic cell surface glycolipids can act as both the primary interface between bacteria and their host and secondly as a targeting mechanism for bacterial virulence factors. The former is characterized by redundancy in adhesin-receptor interactions and the latter by a higher affinity, more restrictive glycolipid binding specificity for targeting. Interactions of verotoxin with its glycolipid receptor globotriaosylceramide and Helicobacter pylori binding to a variety of different glycolipids, which can be environmentally regulated,
provide examples of these differing modes of glycolipid receptor function. Verotoxins are involved in endothelial targeting in the microangiopathies of hemorrhagic colitis and hemolytic uremic syndrome (HUS). The highly restricted binding specificity and crystal structure of the verotoxin B subunit have allowed theoretical modeling of the Gb3 binding site of the verotoxin B subunit pentamer which provides an approach to intervention. Studies of the role of glycolipid function in verotoxin-induced disease have concentrated on the distribution of Gb3 and its ability to mediate the internalization of the toxin within the target cell. The distribution of Gb3 within the renal glomerulus plays a central role in defining the age-related etiology of HUS following gastrointestinal infection with VT producing Escherichia coli. H. pylori, on the other hand, instigates a less distinct but more complex disseminated gastric inflammation. Studies on the role of glycolipid receptors in H. pylori infection have been bogged down in establishing the importance of each binding specificity defined. In addition, the physiological condition of the organism within the various binding assays has not been extensively considered, such that spurious non-physiological interactions may have been elucidated. The identification and cloning of a Le(b) binding adhesin and the identification of cell surface hsp70 as a mediator of sulfoglycolipid binding under stress conditions may now allow a more molecular approach to define the role of glycolipid recognition in this infection.

Llewelyn et al. 2002. Superantigens: microbial agents that corrupt immunity. Lancet Infect Dis. 2(3):156-62. Microbial superantigens are a family of protein exotoxins that share the ability to trigger excessive and aberrant activation of T cells. The best characterised are the staphylococcal enterotoxins and the streptococcal pyrogenic exotoxins that trigger the staphylococcal and streptococcal toxic shock syndromes. It is now apparent that superantigens have a wider role in the pathology of infectious diseases than has previously been appreciated. Staphylococcus aureus and Streptococcus pyogenes together produce 19 different superantigens. The range of microorganisms known to produce superantigens has expanded to include Gram negative bacteria, mycoplasma, and viruses. Research is beginning to shed light on the more subtle parts these molecules play in causing disease and to produce some real possibilities for specific treatment of superantigen-induced toxicity. We aim to highlight these new developments and review the science behind these fascinating molecules.

Lorenz, et al. 1997. In vitro apoptosis and expression of apoptosis-related molecules in lymphocytes from patients with systemic lupus erythematosus and other autoimmune diseases. Arthritis Rheum. 40(2): 306-317. OBJECTIVE: To analyze factors related to apoptosis in systemic lupus erythematosus (SLE) peripheral blood mononuclear cells (PBMC) and to compare the findings in SLE PBMC with those in normal donor PBMC or PBMC from patients with other autoimmune diseases. METHODS: PBMC from normal healthy donors or patients with SLE, mixed connective tissue disease (MCTD), rheumatoid arthritis (RA), or various vasculitides were isolated. The percentage of apoptosis after activation through different signaling pathways was quantified using propidium iodide staining. Protein expression of Fas/APO-1 or bcl-2, and messenger RNA (mRNA) expression of bcl-2, bcl-xL, bax, bak,
Fas/APO-1, Fas ligand (Fas-L), c-myc, mad, or max were determined. RESULTS: We confirmed previous findings of increased numbers of apoptotic cells in SLE PBMC compared with normal donor cells after in vitro incubation. After activation of PBMC with CD28 monoclonal antibody plus phorbol myristate acetate (CD28 MAb/ PMA), staphylococcal enterotoxin B (SEB), or phytohemagglutinin (PHA), the percentage of apoptotic cells was unchanged (SEB) or diminished (CD28 MAb/PMA, PHA) in SLE cells, and the difference between normal donor and SLE cells was less pronounced. On the mRNA level, expression of apoptosis-related gene products did not differ between SLE cells and normal donor cells. Expression of Fas/APO-1 protein was increased in freshly isolated SLE T lymphocytes compared with normal donor T lymphocytes, whereas bcl-2 protein was up-regulated after a 3-day culture period. Cellular activation further increased bcl-2 protein levels, eliminating differences between normal donors and SLE patients. In RA cells, the percentage of apoptosis was similar to that in normal donor PBMC, whereas results using cells from patients with other autoimmune diseases (MCTD, Wegener's granulomatosis, Takayasu arteritis, polyarteritis nodosa) were comparable with those found using SLE PBMC. Addition of growth factors such as interleukin-2 (IL-2), IL-4, or IL-15 to culture medium decreased the percentage of in vitro apoptosis in both normal donor and SLE cells. CONCLUSION: Based on these data, we conclude that accelerated in vitro apoptosis and increased Fas/ APO-1 and bcl-2 protein expression in SLE are nonspecific for the disease, and might be explained at least in part by the increased in vivo activation levels of PBMC from patients with SLE, MCTD, or autoimmune vasculitides combined with in vitro incubation under "noninflammatory" conditions and growth factor withdrawal.

Staphylococcal enterotoxin B (SEB), a primary cause of food poisoning, is also a superantigen that can cause toxic shock after traumatic or surgical staphylococcal wound [correction of would] infections or viral influenza-associated staphylococcal superinfections or when aerosolized for use as a potential biologic warfare threat agent. Intranasal or intramuscular (i.m.) immunization with formalinized SEB toxoid formulated with meningococcal outer membrane protein proteosomes has previously been shown to be immunogenic and protective against lethal respiratory or parenteral SEB challenge in murine models of SEB intoxication. Here, it is demonstrated that immunization of nonhuman primates with the proteosome-SEB toxoid vaccine is safe, immunogenic, and protective against lethal aerosol challenge with 15 50% lethal doses of SEB. Monkeys (10 per group) were primed i.m. and given booster injections by either the i.m. or intratracheal route without adverse side effects. Anamnestic anti-SEB serum immunoglobulin G (IgG) responses were elicited in all monkeys, but strong IgA responses in sera and bronchial secretions were elicited both pre- and post-SEB challenge only in monkeys given booster injections intratracheally. The proteosome-SEB toxoid vaccine was efficacious by both routes in protecting 100% of monkeys against severe symptomatology and death from aerosolized-SEB intoxication. These data confirm the safety, immunogenicity, and efficacy in monkeys of parenteral and respiratory
vaccination with the proteosome-SEB toxoid, thereby supporting clinical trials of this vaccine in humans. The safety and enhancement of both bronchial and systemic IgA and IgG responses by the proteosome vaccine delivered by a respiratory route are also encouraging for the development of mucosally delivered proteosome vaccines to protect against SEB and other toxic or infectious respiratory pathogens.


Mucosally active vaccine adjuvants which will prime a full range of local and systemic immune responses against defined antigenic epitopes are much needed. Cholera toxin (CT) and lipophilic immune stimulating complexes (ISCOMs) containing Quil A can both act as adjuvants for orally administered antigens, but through separate pathways, as evidenced by the dependence on IL-12 for the effect of ISCOMs, whereas CT is independent of this cytokine. Unfortunately the toxicity of CT and recent findings of accumulation of CT in the olfactory nerve and bulb after intranasal administration precludes the clinical use of CT. However, we have been successful in separating the adjuvant and toxic effects of CT, by constructing a gene fusion protein, CTA1-DD, that combines the enzymatically active CTA1-subunit with a B cell targeting moiety, D, derived from Staphylococcus aureus protein A. The present review gives a background to mucosal immunization and the use of -adjuvants in general, followed by a description of a strategy to rationally design a vaccine adjuvant vector that fulfils the criteria of targeting and immunomodulating innate immunity in order to boost a strong adaptive immune response. We have combined CTA1-DD and ISCOMs into a new highly promising vaccine adjuvant vector, CTA1-DD/ISCOMs. The combined vector is immunogenic when given by the subcutaneous, oral or nasal routes, inducing strong cell--mediated and humoral immune responses, including local mucosal IgA. It requires the ADP ribosylating property of the CTA1-enzyme and the effect of the combined vector greatly exceeded the effect of either ISCOMs or CT used alone. Antigens could be incorporated into or just admixed with the new vector. Thus, we have demonstrated that rationally designed vectors consisting of CTA1-DD and ISCOMs may provide a novel strategy for the generation of potent and safe mucosal vaccines.

Lycke. 2001. The B-cell targeted CTA1-DD vaccine adjuvant is highly effective at enhancing antibody as well as CTL responses. Curr.Opin.Mol.Ther. 3(1): 37-44.

A novel immunomodulating gene-fusion protein, CTA1-DD, has been developed which combines the ADP-ribosylating ability of cholera toxin (CT) with a dimer of an Ig-binding fragment, D, of Staphylococcus aureus protein A. The CTA1-DD adjuvant is non-toxic and greatly augmented T-cell-dependent and T-cell-independent responses to soluble admixed antigens after systemic as well as mucosal immunizations. CTL and antibody responses of all classes were increased by 10- to 100-fold above those observed in control mice immunized without adjuvant. CTA1-DD does not appear to form immune complexes or bind to soluble Ig following injection, but rather it binds directly to B-cells of all isotypes, including naive IgD+ cells. No binding was observed to macrophages or dendritic cells, and immunizations in Fc gamma R-deficient mice demonstrated unaltered enhancing effects. As shown by inactive mutants, the CTA1-DD adjuvant is dependent
on ADP-ribosyltransferase activity and requires the binding to Ig- via the DD moiety. The enhancing effect is associated with enlarged germinal centers, and binding of CTA1-DD to the B-cells strongly upregulates co-stimulatory molecules and counteracts apoptosis by inducing intracellular Bcl-2.


Matrix metalloproteinases (MMP) are a family of structurally related endopeptidases that resorb macromolecules of the extracellular matrix (ECM). They are involved in normal tissue remodeling and wound repair as well as in pathological processes such as the irreversible destruction of joints observed in rheumatoid arthritis (RA). In addition, MMP catalyze the cleavage of the transmembrane form of tumor necrosis factor (TNF). Since cells of the monocyte lineage are major producers of TNF in the rheumatoid synovium we analyzed the expression of MMP genes in these cells. To examine the transcriptional activity of MMP genes in undifferentiated monocytic cell lines (MonoMac6, U937) and in nature human monocytes isolated from peripheral blood, we developed an assay that is based on reverse transcription (RT) followed by a polymerase chain reaction (PCR). This screening procedure demonstrates that several MMP genes are transcriptionally active in the cells tested after exposure to a variety of stimuli such as phorbol ester, lipopolysaccharide (LPS) and staphylococcal enterotoxin B (SEB). The data were confirmed by quantitative Northern blot analysis. In conclusion, cells of the monocyte lineage produce high mRNA levels of at least six members of the MMP gene family that could participate in joint destruction by resorption of the ECM and secretion of TNF.


The pathology of aerosolized staphylococcal enterotoxin B (SEB) was studied in the nonhuman primate. Six juvenile rhesus monkeys that received multiple lethal inhaled doses of SEB developed diarrhea and vomiting within 24 hr followed by depression, dyspnea, and shock. Three of 6 animals died by 52 hr. The most striking gross lesion in all 6 monkeys was diffuse severe pulmonary edema. Histologically, edema fluid was present within the peribronchiolar, peribronchial, and perivascular interstitium, alveolar septa, and alveoli. The adventitia of pulmonary vessels was infiltrated by lymphocytes, macrophages, and fewer neutrophils. Numerous large lymphocytes with occasional mitotic figures were within pulmonary vessels, often occluding alveolar capillaries. These cells were strongly immunoreactive with monoclonal antibodies against CD3, establishing them as T cells. Ultrastructurally, endothelial cell junctions were intact, and endothelial cells and type I pneumocytes contained numerous pinocytotic vesicles. Alveolar septal interstitial spaces were expanded by edema. The mechanism of these SEB-induced pulmonary lesions was not determined. We hypothesize that cytokine production by activated T cells may have caused vascular permeability changes leading to widespread pulmonary edema and shock.
Members of the Bacillus genus are ubiquitous soil microorganisms and are generally considered harmless contaminants. However, a few species are known toxin producers, including the foodborne pathogen, B. cereus. This species produces two distinct types of foodborne illness, the emetic (vomit-inducing) syndrome, associated with consumption of toxin in cooked rice dishes, and the diarrheal illness seen occasionally following consumption of contaminated meats, sauces, and certain dairy products. In the latter case, illness results from the production of enterotoxins by vegetative cells in the small intestine of the host. In dairy products, the occurrence of Bacillus spp. is inevitable, and the spore-forming ability of this organism allows it to easily survive pasteurization. Many strains have been shown to grow and produce enterotoxin in dairy products at refrigeration temperatures. Evaluation of toxin gene presence and toxin expression in Bacillus spp. other than B. cereus has not been thoroughly investigated. However, the presence of natural isolates of Bacillus spp. harboring one or more enterotoxin gene(s) and subsequent demonstration of conditions which may support toxin expression holds crucial importance in the food safety arena.

Although autoreactive T cells are thought to play a prominent role in autoimmune disease in MRL-lpr/lpr mice, it has been difficult to directly determine if autoreactive T cells escape from the thymus and react with self-antigens in the periphery. To identify a possible defect in clonal deletion or clonal anergy induction of auto-specific T cells, we have studied C57BL/6-lpr/lpr transgenic mouse expressing TCR genes that recognize a known self-antigen, the male H-Y antigen and analyzed clonal deletion and tolerance induction after neonatal tolerance induction with the class II MHC reactive superantigen staphylococcal enterotoxin B (SEB) in V beta 8 TCR transgenic and non-transgenic MRL-lpr/lpr mice. In lpr/lpr mice, the main defect was a thymic-dependent loss of self-tolerance by auto-specific T cells and also a small defect of clonal deletion of autoreactive thymocytes. Defective expression of the Fas apoptosis antigen results from the insertion of the ETn retrotransposon. The fas defect can be partially corrected in TCR-beta TCR transgenic mice in which accelerated T cell development prevents the lymphoproliferative disease and in CD2-fas transgenic mice in which fas expression is corrected in T cells. These results suggest that expression of the TCR-beta, fas and retrovirus genes are co-regulated during early thymocyte development, most likely by common enhancer transcription factors.

Although autoreactive T cells are thought to play a prominent role in autoimmune disease in MRL-lpr/lpr mice, it has been difficult to directly determine if autoreactive T cells escape from the thymus and react with self-antigens in the periphery. Defective expression of the Fas apoptosis antigen in MRL-lpr/lpr mice results from the insertion of the ETn retrotransposon. The fas defect can be partially corrected in CD2-fas transgenic
mice in which the expression of fas is corrected in T cells. To identify a possible defect in clonal deletion or clonal anergy induction of auto-specific T cells, we have studied C57BL/6-lpr/lpr transgenic mice that express TcR genes that recognize a known self-antigen, the male H-Y antigen. In addition, we have analyzed clonal deletion and tolerance induction after neonatal tolerance induction and superantigen-induced arthritis with the class II MHC reactive superantigen staphylococcal enterotoxin B (SEB) in V beta 8 TcR transgenic and non-transgenic MRL-lpr/lpr mice. Neonatal tolerance induction to SEB was normal in lpr/lpr mice. However, over time a loss of tolerance (thymic or peripheral) was observed in lpr/lpr mice but not in +/- TcR transgenic mice. This defect in lpr/lpr mice was thymic-dependent and was due to increased CD28/CTLA4 signaling. These results suggest that an apoptosis defect involving both thymocytes and peripheral lymphoid cells leads to autoimmune disease in lpr/lpr mice. The challenge in the future will be to determine the role of defective apoptosis in other autoimmune diseases.


MRL-lpr/lpr mice are defective in the fas Ag/APO-1 apoptosis gene (CD95). Using the hepatotoxin D-galactosamine (D-GalNH2), we demonstrate that MRL-lpr/lpr mice have an increased susceptibility to staphylococcal enterotoxin B (SEB)-induced lethal shock, which causes them to exhibit the septic shock-like behaviors of fur ruffling and listlessness, and death occurs within 8 to 18 h. SEB susceptibility is greater in V beta 8.2 TCR transgenic MRL-lpr/lpr mice than in nontransgenic mice. In studies designed to elucidate the molecular pathways of SEB-induced septic shock, we found that C57Bl/6.Ab0/Ab0, MHC class II-deficient "C2D" mice, but not C57Bl/6-(+/-) mice, are nonresponsive to challenge with SEB. C2D mice, backcrossed with the fas mutation resulting in double-knockout C2D:lpr/lpr mice, are more susceptible to challenge with SEB/D-GalNH2. The LD50s for C57Bl/6.C3H- gld/gld "fas ligand-mutant mice" challenged with SEB/D-GalNH2 were comparable to C57Bl/6.MRL-lpr/lpr and MRL-lpr/lpr mice, suggesting that reciprocal mutations in either fas or fas ligand increases susceptibility to bacterial superantigens (SAGs). SEB-induced lethal shock can be reversed by treatment with Abs to V beta 8 TCR, MHC class II Ia+, IL-2, and TNF-alpha, by the immunosuppressant cyclosporin A, or by treatment with carbocyclic nucleoside analogues. These data indicate that SAG-induced septic shock is dependent on interactions with the TCR and MHC class II Ags, and they also suggest a critical role for a functional fas and/or fas ligand in resistance to SAG-induced septic shock.


Subcutaneous injection of Staphylococcal enterotoxin B (SEB) produced by Staphylococcus aureus, caused severe arthritis in DBA/1J mice which had been previously immunized with bovine type II collagen. The severity of this arthritis was dose dependent and prolonged joint inflammation with erosion of bone was observed. Anti-
type II collagen antibodies were detected in the serum of arthritic mice. Effector T cells against type II collagen were also detected by means of delayed type hypersensitivity in the skin. Moreover, a significant decrease in the ratio between T cells and B cells and an increase in the ratio between CD4+ cells and CD8+ cells was observed in spleen cells from arthritic mice. Prednisolone suppresses the induction and development of clinical signs of arthritis in mice. This evidence suggests that this experimental arthritis model may provide a means to examine the role of superantigens and the efficacy of pharmacological agents for the treatment of rheumatoid arthritis.

The effect of a novel thiazole derivative, SM-8849, on experimental arthritis in mice was studied and compared to that of prednisolone. SM-8849 and prednisolone reduced the incidence and severity of type II collagen-induced arthritis in mice, as assayed by clinical observation and histopathological studies. Although both agents inhibited type II collagen-induced delayed type hypersensitivity (DTH) in arthritic mice, SM-8849 did not affect the production of humoral antibodies to type II collagen. To examine the inhibitory mechanism of SM-8849, the effects on T cell-dependent allergic inflammation were studied. SM-8849 clearly inhibited T cell-dependent reactions including staphylococcal enterotoxin B (SEB)-induced arthritis, SEB-induced CD25 expression on T cells and sheep red blood cell (SRBC)-induced DTH reaction. SM-8849, however, had no effect on the production of humoral antibody forming cells in the spleen of mice immunized with SRBC. These results indicate that inhibition of type II collagen-induced arthritis by SM-8849 is mainly due to the inactivation of T cells that are related to DTH reaction.

The effects of mesoporphyrin, a novel porphyrin derivative, on type II collagen-induced arthritis in mice were studied. Mesoporphyrin (10-30 mg/kg) and prednisolone (5 mg/kg; reference drug) reduced the incidence and severity of type II collagen-induced arthritis in mice, as assayed by clinical observation and histopathological studies. Although both agents inhibited type II collagen-induced delayed type hypersensitivity in arthritic mice, only prednisolone inhibited humoral immunity to type II collagen. The effects of mesoporphyrin on T cell dependent allergic inflammation were examined, in order to study the mechanism by which it inhibits arthritis. Staphylococcal enterotoxin B (SEB; superantigen)-potentiated collagen-induced arthritis and sheep red blood cell-induced delayed type hypersensitivity reaction were clearly inhibited by mesoporphyrin. Moreover, the superantigen-induced CD-25 expression on T cells was inhibited by mesoporphyrin. These results indicate that mesoporphyrin inhibits type II collagen-induced arthritis by inhibiting the activation of T cells.

Although the immunosuppressive properties of anti-CD3 mAbs are now widely
recognized, we have accumulated data characterizing the T cell activating properties of these antibodies. While in some situations these activating properties may be viewed as unwanted side-effects (for instance OKT3-mediated T cell activation may be responsible for some of the first dose toxicity seen with patients receiving OKT3 for suppression of allograft rejection), we have shown that anti-CD3 mAb therapy can augment host immune responses and provide protection against some tumors and viral infections. Importantly, this augmented response allows the development of long term, specific immunity. Because the immunosuppressive and activating properties of anti-CD3 mAbs are so closely overlapping, we have sought to identify other agents that are capable of activating T cell subsets selectively. We have found that SEB activates T cell subsets selectively in vivo and that this activation can be exploited to prevent the outgrowth of a malignant murine tumor. Studies currently in progress, including phenotypic and functional analysis of TILs and in vivo T cell subset depletions, should result in a more precise understanding of how SEB-induced T cell activation inhibits tumor growth.


The repeated injection of bacterial superantigens (SAg), such as staphylococcus enterotoxin (SE) A or B, has been shown in mice to induce a state of unresponsiveness characterized by the lack of secretion of Th1 lymphokines, such as IL-2 and IFN-gamma, following subsequent SAg challenge. We made the observation, in vivo as well as in vitro, that unresponsiveness to SAg could be transferred from SEA- to SEB-reactive T cells (and reversibly from SEB- to SEA-specific T cells) in C57BL/6 mice but not in BALB/c mice. Since C57BL/6 mice, unlike BALB/c mice, possess TCR V(beta)3+ and V(beta)11+ T cells able to react with both SEA and SEB, we hypothesized that SAg-unresponsive V(beta)3(+) and V(beta)11+ T cells could mediate linked suppression of other SAg-reactive T cells. To analyze further this possibility, spleen cells from BALB/c mice made unresponsive to SEB were tested for their capacity to suppress the response of normal BALB/c cells to SEB. The production of both IFN-gamma and IL-2 following SEB stimulation was greatly impaired in co-cultures containing CD4(+) T cells, but not CD8(+) T cells, isolated from unresponsive animals. In vivo, the production of both IFN-gamma and IL-2 responses to SEB was dramatically reduced in animals adoptively transferred with unresponsive spleen cells. This suppression was abrogated in recipients injected with neutralizing anti-IL-10 antibodies. Moreover, in animals made unresponsive to SEB, SAg-reactive CD4(+) T cells were found to express high levels of CTLA-4, a molecule recently described to play an essential role in the suppressive function of regulatory T cells. Taken together these results demonstrate that the repetitive injection of SAg induces the differentiation of regulatory CD4(+) T cells capable of suppressing SAg-reactive naive T cells.


The accessory genes of Staphylococcus aureus, including those involved in pathogenesis, are controlled by a complex regulatory network that includes at least four two-component systems, one of which, agr, is a quorum sensor, an alternative sigma factor and a large set
of transcription factors, including at least two of the superantigen genes, tst and seb. These regulatory genes are hypothesized to act in a time- and population density-dependent manner to integrate signals received from the external environment with the internal metabolic machinery of the cell, in order to achieve the production of particular subsets of accessory/virulence factors at the time and in quantities that are appropriate to the needs of the organism at any given location. From the standpoint of pathogenesis, the regulatory agenda is presumably tuned to particular sites in the host organism. To address this hypothesis, it will be necessary to understand in considerable detail the regulatory interactions among the organism's numerous controlling systems. This review is an attempt to integrate a large body of data into the beginnings of a model that will hopefully help to guide research towards a full-scale test.


We have distinguished TSST-1 from SEA and SEB with respect to its in vivo effect on T cells, that is, SEA and SEB induce tolerance in treated mice whereas injection of TSST-1 does not result in tolerance. Therefore, previous observations which relate superantigens to the suppression of reactive V beta TCR T cells seem difficult to generalize to all bacterial superantigens. Since the effects of superantigens are beginning to be exploited for immunotherapy, the differences between TSST-1 and SEB in terms of induction of tolerance suggest that all superantigens may not generally be useful in this respect. Each superantigen obviously should be studied in vivo in respect to T cell tolerance induction.


Recently, in Japan newly neonatal exanthematous disease was elucidated to be caused by staphylococcal superantigenic exotoxins, mainly TSST-1. We studied exotoxins producibility of 43 strains of S. aureus isolated from neonates with exanthematous disease and examined antibody titers to staphylococcal enterotoxin A, B, C (SEA, SEB, SEC) and toxic shock syndrome toxin 1 (TSST-1) of the patients and control (umbilical cord blood from term infants). The results were as follows: 1. 34 of 43 strains (79%) isolated from the patients were SEC and TSST-1 producing MRSA, 5 strains (12%) were SEB, SEC, and TSST-1 producing MRSA, 1 strain (2%) was SEB and TSST-1 producing MRSA, 2 strains (12%) were SEB producing MSSA and did not produce TSST-1. The 1 strain (2%) was MSSA which produced SEC and TSST-1. 2. 16 neonates with exanthematous disease, who showed typical clinical signs and laboratory findings of thrombocytopenia, with SEC and TSST-1 producing MRSA isolates had significantly low anti-TSST-1 antibody titers at onset (p < 0.05), compared with the control (umbilical cord blood from term infants): TSST-1 appeared to the causative agent for the disease. In two neonates with exanthematous disease, with SEB- and non- TSST-1-producing MSSA isolates, anti-SEB antibody titers were low at onset, so SEB appeared to be the causative agent for the disease. 3. In Japan, low anti-TSST-1 antibody titers were found in the umbilical blood samples from about 70% of term infants; and low anti-SEB or anti-SEC
antibody titers were found in samples from only about 10% of them, that is, a number of term infants had anti-SEB and anti-SEC antibodies. The majority of S. aureus isolated from neonates with exanthematous disease were enterotoxin- and TSST-1-producing MRSAs. The results of our study by measuring antitoxin antibody titers suggested that SEB and SEC might not be pathogenically responsible, but TSST-1 was considered to be responsible for the majority of exanthematous disease. Prevalence of TSST-1-producing MRSA in the neonatal and premature baby ward is the main cause for the high incidence of this disease in Japan, whereas the low antibody titer to TSST-1 in the mother, in comparison with the anti-enterotoxin antibody titers, may also be a predisposing factor.

OBJECTIVE: To investigate the mechanism of autoimmune phenomena, occasionally seen in patients with rheumatoid arthritis treated with bucillamine (BUC) and D-penicillamine (D-Pen), by evaluating their effects on apoptosis of T cells induced by T cell receptor activation or dexamethasone. METHODS: In vitro apoptosis was induced in a T cell hybridoma (SSP3.7) and a B cell line (WEHI 231) by activation of respective receptors or dexamethasone, in the presence or absence of BUC or D-Pen. In vivo apoptosis was induced in BALB/c mice by staphylococcal enterotoxin B (SEB), with or without BUC or D-Pen, and thymocytes were examined for it by FACS. RESULTS: Stimulation with anti-CD3 and dexamethasone induced apoptosis in 72% and 71% of SSP3.7 cells, respectively. However, only 16% of SSP3.7 cells became apoptotic by anti-CD3 when BUC was added to the culture media. By contrast, 80% of SSP3.7 cells became apoptotic when stimulated by dexamethasone, even in the presence of BUC. BUC did not affect apoptosis of WEHI 231 cells induced by anti-IgM. Although SA981 (a metabolite of BUC) inhibited apoptosis of SSP3.7 cells induced by anti-CD3, D-Pen did not. BUC, SA981, or D-Pen did not significantly influence the level of interleukin 2 secretion stimulated by anti-CD3. In contrast, both BUC and D-Pen inhibited apoptosis of Vbeta8+ thymocytes induced in vivo by SEB superantigen. Neither BUC nor D-Pen significantly changed the number of CD4+CD8+ thymocytes in BALB/c mice injected with dexamethasone. CONCLUSION: BUC decreased, while D-Pen did not, the apoptosis of T cells stimulated by anti-CD3 in vitro, although they both inhibited the deletion of immature thymocytes reactive with SEB in vivo. This may explain autoimmune phenomena sometimes seen during the treatment of rheumatic patients with these drugs.

OBJECTIVES: To study recombinant human tissue factor pathway inhibitor (rhTFPI) in a superantigen-induced shock model and in a cecal ligation and puncture (CLP) model of peritonitis in mice. DESIGN: Prospective, randomized, experimental study. SETTING: An experimental animal research laboratory. SUBJECTS: Eighty BALB/c mice for the superantigen model, and 56 BALB/c mice for the CLP model. INTERVENTIONS: In the superantigen-induced shock model, animals received rhTFPI (350 mg/kg) subcutaneously
every 12 hrs (n = 30) or saline control (n = 30) for 60 hrs after staphylococcal enterotoxin B (SEB; 10 microg iv) and a sublethal dose of E. coli 0111:B4 lipopolysaccharide (LPS; 75 microg ip). Control groups received SEB alone (n = 10) and LPS alone (n = 10). In the CLP model, rhTFPI or saline was given every 8 hrs for 48 hrs by using a 21-gauge needle (n = 9) or 23-gauge needle (n = 14) for CLP. A sham surgery control group (n = 10) was also included. MEASUREMENTS AND MAIN RESULTS: There was 0% mortality in the SEB and LPS control groups. The mortality rate was 64% in the saline control group that received both SEB and LPS (19 of 30), whereas the rhTFPI- treated animals had a mortality rate of 20% (6 of 30; p < .01). The rhTFPI-treated group had significantly lower interleukin-6 levels (61.8 +/- 41 pg/mL vs. 285 +/- 63 pg/mL; p < .05) than the control group but no differences in tumor necrosis factor-alpha or interferon-gamma levels. In the CLP experiment, rhTFPI-treated animals did not have any survival advantage over the control group after the large-bore (21-gauge) needle puncture. The rhTFPI group had significantly improved 7-day mortality rate after CLP with the small-bore needle (23-gauge; 21.4% [rhTFPI] vs. 71.4% [control], p < .01). Plasma LPS, interleukin-6, interferon-gamma, and tumor necrosis factor-alpha levels were unchanged by rhTFPI treatment, but significantly reduced LPS (p = .006) and IFNgamma (p = .001) levels were found in the peritoneal fluid. CONCLUSIONS: Tissue factor pathway inhibitor significantly improves the mortality rate in models of superantigen-induced shock and polymicrobial intra-abdominal infection, supporting its potential use in clinical trials for septic shock.

Origuchi et al. 1995. Increased levels of serum IgM antibody to staphylococcal enterotoxin B in patients with rheumatoid arthritis. Ann Rheum Dis. 54(9):713-20. OBJECTIVE--To investigate the role of superantigen in rheumatoid arthritis (RA) by assaying the serum levels of staphylococcal enterotoxin B (SEB) antibodies. METHODS-Serum IgG and IgM SEB antibodies were measured using an enzyme linked immunosorbent assay (ELISA), and confirmed by Western blot analysis. The T cell receptor V beta (TCR V beta) repertoire was analysed using the reverse transcriptase polymerase chain reaction. RESULTS--RA patients had increased levels of serum IgM SEB antibody compared with normal subjects, patients with systemic lupus erythematosus, Sjogren's syndrome, and Behcet's disease. The titres of rheumatoid factor (RF) showed no correlation with the levels of IgM SEB antibodies, and the levels of SEB antibodies were not inhibited by the addition of human immunoglobulin, or after absorption of RF. RA patients whose disease duration was less than 10 years had greater levels of serum IgM SEB antibodies than those with disease duration more than 10 years. The levels of IgM and IgG SEB antibodies in synovial fluid from RA patients were correlated with those in their sera. Western blot analysis detected IgM and IgG SEB antibodies as a band of approximately 30 kDa molecular size. The percentage of TCR V beta 2, V beta 5.2, and V beta 12 in phytohaemagglutinin stimulated peripheral T cells correlated significantly with the levels of serum IgM SEB antibody in RA patients. CONCLUSION--These results suggest that SEB, one of the superantigens, may have a critical role in the pathogenesis of RA.

**PURPOSE:** The purpose of this study was to determine the optimal conditions for prolonging corneal allograft survival by inducing anergy with the superantigen staphylococcal enterotoxin B (SEB). **METHODS:** A rat model of penetrating keratoplasty, whereby Fisher344 donor corneas are implanted into Lewis recipients, was used to evaluate the effects of SEB on inhibiting immune-mediated allograft rejection. To induce anergy, SEB was injected into the peribulbar space of Lewis rats. Furthermore, histopathology and immunofluorescent staining were used to examine the levels of infiltrating CD4(+) and CD8(+) T lymphocytes and NK1.1(+) lymphocytes. **RESULTS:** By administering SEB, at doses of 90 or 120 micro g/kg 7 days before and after keratoplasty, we suppressed the episode of corneal graft rejection for a median of 12 and 30 days, respectively. In contrast, rejection was observed when 30 or 60 micro g/kg of SEB was administered. After SEB injections, lymphocyte infiltration into the corneal grafts was reduced, and the expression of NK1.1(+) lymphocytes was enhanced, suggesting that anergy may be occurring. Also, there were no differences in the number of infiltrating CD4(+) and CD8(+) T lymphocytes cells between the control group and groups injected with 30 and 120 micro g/kg SEB on postoperative days 10 and 30. **CONCLUSIONS:** Inducing anergy with the superantigen SEB prolonged corneal graft survival in a rat model of penetrating keratoplasty. Therefore, these results support the possibility of prolonging corneal allograft survival in a clinical setting by preventing immune-mediated rejection through the administration of the superantigen SEB.


Staphylococcal enterotoxin B is a member of a family of toxins known as superantigens that activate a large number of T-cells (up to 20%) by cross-linking MHC class II molecules with T-cell receptors in a Vbeta-restricted fashion. The crystal structure of staphylococcal enterotoxin B presented here has been determined at 1.5 A resolution, the highest resolution so far for a superantigen. The final model contains 1948 protein atoms and 177 water molecules and has excellent geometry with root-mean-square (rms) deviation of 0.007 A and 1.73 degrees in bond lengths and bond angles, respectively. The overall fold is similar to that of other microbial superantigens, but as it lacks the zinc-binding site found in other members of this family, such as staphylococcal enterotoxin A, C2 and D, this enterotoxin possesses only one MHC class II binding site. Comparison of the crystal structure of free SEB and in complex with an MHC class II molecule revealed no major changes in the MHC-binding site upon complex formation. However, a number of water molecules found in the free SEB may be displaced in the complex or contribute further to its stability. Detailed analysis of the TcR-binding site of SEB, SEA and SEC2 shows significant differences which may account for the ability of each superantigen to bind specific Vbeta sequences. Copyright 1998 Academic Press Limited.

We have achieved sensitive, rapid and reproducible detection of three biological threat agents in a variety of biological and environmental matrices using the DELFIA time-resolved fluorometry (TRF) assay system (Perkin-Elmer Life Sciences, Akron, OH). Existing ELISA assays for the detection of Francisella tularensis, Clostridium botulinum A/B neurotoxin (BotNT A/B), and Staphylococcus aureus enterotoxin B (SEB) were converted to TRF assays. They use 100 microl of positive control or unknown per test well and require just over 2 h to run. Fluorescent signal read time is a fraction of a second per well. The assay format consists of a capture ELISA utilizing a biotinylated capture antibody, prebound to a streptavidin-coated 96-well plate and a lanthanide (Europium, Eu3+)-labeled detector antibody. The bound Eu-labeled detector antibody produces a fluorescent signal upon the addition of an enhancement solution. The signal results from the dissociation of the Europium from the antibody, creating a micelle, thus amplifying the signal nearly one million-fold. Sensitivities achieved by these assays were between 4 and 20 pg/ml in buffer. Additionally, we have tested this system in different matrices such as serum, urine, dirt, and sewage. Concentration curves generated from standard solutions produced a wide linear range making serial dilutions of unknown samples unnecessary. DELFIA TRF assays are significantly better in terms of sensitivity, linear range, and run time than standard capture ELISAs and should facilitate early detection of potential biological warfare agents in clinical and environmental samples.


One of the leading causes of death for women is metastatic breast cancer. Because most animal tumors do not accurately model clinical metastatic disease, the development of effective therapies has progressed slowly. In this study, we establish the poorly immunogenic mouse 4T1 mammary carcinoma as a postsurgical animal model. 4T1 growth characteristics parallel highly invasive human metastatic mammary carcinoma and, at the time of surgery, the extent of disease is comparable with human stage IV breast cancer. Progress in understanding the immune response has led to innovative immune-based anticancer therapies. Here, we test in this postsurgical model, a novel cell-based vaccine, combining MHC class II, CD80(B7.1), and SEB superantigen. Effective treatment of tumor-bearing mice with this immunotherapy requires expression of all three molecules. Mean survival time is extended from 5-7.5 weeks for control-treated mice to 6-10.5 weeks for therapy-treated mice. Increased survival is accompanied by a maximum of 100-fold decrease in clonogenic lung metastases. These therapeutic effects are particularly noteworthy because: (a) the postoperative model demonstrates that early metastases responsible for morbidity are established by 2 weeks after tumor inoculation with 7 x 10(3) parental 4T1 cells into the mammary gland; (b) the immunotherapy is
started 4 weeks after tumor inoculation when the mice contain extensive, pre-established, disseminated metastases; and (c) CD4+ and CD8+ T cells are required for the effect.

We have developed a model of peripheral in vivo T cell tolerance that is induced by administration of the protein superantigen staphylococcal enterotoxin B (SEB). Rather than activating V beta 8+ T cells, in vivo administration of SEB induced in them a profound state of anergy. This was shown by their failure to proliferate to subsequent in vitro restimulation with SEB or to anti-V beta 8 antibodies. This unresponsiveness was V beta 8 specific since T cells from SEB-immunized mice responded normally to other antigens. 8 d after SEB administration, there was no reduction in the number of V beta 8+ T cells or in the intensity of V beta 8 T cell receptor (TCR) expression. Although a portion of the V beta 8+ T cells from SEB-primed mice were able to express interleukin 2 receptors (IL-2Rs), they failed to proliferate in response to exogenous IL-2, indicating they were defective in their IL-2 responsiveness. 2-4 wk after SEB administration, there was a reduction of approximately 50% in the number of V beta 8+ cells in immunized compared with control animals. There was, however, no reduction in the level of TCR expression on the remaining V beta 8+ cells. These data demonstrate that proteins that activate T cells in vitro in a V beta-specific manner can induce a state of anergy in peripheral T cells in vivo and may possibly further mediate clonal deletion in a portion of the tolerized cells.

Most pathogenic microorganisms enter their host via the mucosal surfaces lining the digestive, respiratory and urino-reproductive tracts of the body. The most efficient means of protecting these surfaces is through mucosal immunization. Transgenic plants are safe and inexpensive vehicles to produce and mucosally deliver protective antigens. However, the application of this technology is limited by the poor response of the immune system to non-particulate, subunit vaccines. Co-delivery of therapeutic proteins with targeting proteins, such as the B subunit of the Escherichia coli heat labile enterotoxin (LTB), could increase the effectiveness of such antigens.

Hepatitis C virus (HCV) is the leading cause of chronic hepatitis, affecting approximately 2% of the world's population. The immune mechanisms responsible for the highly variable natural history in a given individual are unknown. We used a multiparameter flow cytometric technique to functionally and phenotypically characterize HCV-specific effector T cells in the peripheral blood of 32 individuals with different stages of hepatitis C disease (resolved, mild chronic, advanced chronic) and normal controls. We found the highest frequencies of virus-specific effector cells with an activated memory phenotype (CD45RO+CD69+) in subjects who had resolved HCV infection, either spontaneously or with antiviral therapy. Effector cells from patients with resolved infection produced Th1
type cytokines following stimulation with nonstructural antigens (NS3 and NS4), whereas effector cells from chronically infected patients produced Th1 type cytokines predominantly following stimulation with the HCV core antigen. Stimulation with superantigen staphylococcal enterotoxin (SEB) induced the same levels of cytokine production in the different patient groups. Among the HCV-seropositive patients, viral load inversely correlated with the Th1 effector cell response to NS3. Interleukin (IL)-4 was produced only in response to the control antigens, but not in response to the HCV recombinant proteins. Taken together, these findings suggest that a vigorous HCV-specific CD4+ Th1 response, particularly against the nonstructural proteins of the virus, may be associated with viral clearance and protection from disease progression. Prospective studies using this new flow cytometric assay will be required to determine whether antiviral therapy modifies the frequency, specificity, and function of these virus-specific effector cells.


The objective of this article is to provide a concise overview of the most likely biological and chemical agents that could be used as biochemical weapons. The diagnosis, pathology, prevention, decontamination, treatment, and disposition of these biological and chemical agents are presented in a tabular format for quick reference purposes. The information provided outlines the bare essentials needed to deal with any emergency or catastrophic event involving these agents.


**BACKGROUND:** Enterotoxins produced by Staphylococcus aureus and their specific IgE antibodies were thought to be important in worsening atopic dermatitis. However, few studies have documented an association between *S.* aureus or its exotoxins and exacerbations of upper airway/nasal disease. In the current study, we determined the prevalence of serum-specific IgE towards staphylococcal enterotoxin A, B, C, D (SEA, SEB, SEC, SED) and toxic shock syndrome toxin 1 (TSST-1) in patients suffering from rhinitis and/or asthma due to allergy. Therefore, we examined whether SEA, SEB, SEC, SED and TSST-1 were important in worsening the clinical status of patients allergic to house dust mites by means of assessing serum eosinophil cationic protein (ECP), which is thought to be a reliable marker of asthma and rhinitis severity. **METHODS:** 198 patients with persistent allergic rhinitis and/or asthma due to house dust mites were evaluated. Specific IgE towards SEA, SEB, SEC, SED, TSST-1, timothy grass and birch pollen recombinant allergens, and other aeroallergen extracts from common allergen sources were evaluated by the Pharmacia CAP System. Serum ECP was assessed, too.

**RESULTS:** The percentages of sensitization to staphylococcal enterotoxins of 198 house dust mite-allergic patients were as follows: TSST-1-specific IgE 24.7% (n=49), SEC-specific IgE 22.2% (n=44), SEB-specific IgE 15.1% (n=30), SEA-specific IgE 9.1% (n=18), and SED-specific IgE 5.5% (n=11). Out of 198 individuals allergic to house dust mites 136 patients suffering from persistent rhinitis were subdivided into two subgroups:
53 patients with serum-specific IgE to at least one staphylococcal enterotoxin and 83 patients without specific IgE towards staphylococcal enterotoxins. Patients sensitive to staphylococcal enterotoxins had higher serum ECP levels than patients lacking specific IgE to SEA, SEB, SEC, SED and TSST-1 (geometric mean 24.3 vs. 16.6 microg/100 ml; p=0.029), as well as total IgE levels (geometric mean 564 vs. 161 kU/l; p=0.00063) and specific IgE to Dermatophagoides pteronyssinus (geometric mean 16.7 vs. 6.6 kU/l; p=0.0235) and Dermatophagoides farinae (geometric mean 18.6 vs. 7.8 kU/l; p=0.0246).

CONCLUSION: A status of sensitization to staphylococcal enterotoxins seems to be a factor increasing serum ECP, which is thought to be a reliable marker of clinical severity of allergic disease. Therefore, the evaluation of SEA, SEB, SEC, SED and TSST-1-specific IgE antibodies may have additional significance for the prognosis of persistent allergic diseases of the upper airway.

A fluorescence-based immunosensor has been developed for simultaneous analysis of multiple samples. A patterned array of recognition elements immobilized on the surface of a planar waveguide is used to "capture" analyte present in samples; bound analyte is then quantified by means of fluorescent detector molecules. Upon excitation of the fluorescent label by a small diode laser, a CCD camera detects the pattern of fluorescent antigen:antibody complexes on the sensor surface. Image analysis software correlates the position of fluorescent signals with the identity of the analyte. This immunosensor was used to detect physiologically relevant concentrations of staphylococcal enterotoxin B (SEB), F1 antigen from Yersinia pestis, and D-dimer, a marker of sepsis and thrombotic disorders, in spiked clinical samples.

PROBLEM: Local application of non-replicating antigens to the female reproductive tract is ineffective in stimulating the common mucosal immune system, and induces only weak genital antibody responses. Studies of immune responses to genital infections such as gonorrhea also support the concept that, lacking mucosal immune inductive sites, the reproductive tract is ill-equipped to mount effective immune responses. METHOD OF STUDY: Intranasal (i.n.) and intravaginal (i.vag.) routes of immunization of mice with a protein antigen coupled to cholera toxin (CT) B subunit, or genetically engineered as chimeric proteins with the A2/B sunbunits of CT or type II heat-labile enterotoxin, were compared for their ability to induce specific antibody responses in vaginal fluids, saliva, and serum. RESULTS: Mice immunized i.n. developed substantially stronger vaginal immunoglobulin A (IgA) and immunoglobulin G (IgG) and serum IgG and IgA antibodies, than those immunized i.vag. which also failed to develop salivary antibodies. Vaginal antibody responses induced i.n. persisted for at least 1 year, and were recallable by booster immunization after a prolonged period. CONCLUSIONS: Such alternative strategies for inducing potent genital antibody responses offer the prospect of prophylactic immunization against genital infections. Further studies are required to
evaluate their applicability to humans, and to comprehend the cellular and molecular mechanisms involved in delivering effective immune responses to the reproductive tracts.


Human TCR gamma delta positive T cells can proliferate in response to stimulation with staphylococcal enterotoxins (SEs) or mediate lysis of SE pulsed target cells. In the small number of studies reported, the proliferative response of gamma delta T cells was limited to V gamma 9 negative cells and, in vitro, such responses do not require the presence of MHC class II molecules for antigen presentation. Proliferative responses have been found after stimulation with SEA, SEB and TSST. The cytolytic activity of gamma delta T cells can be mediated by two different mechanisms: either gamma delta T cells specifically interact with SEA pulsed target cells--this is most likely TCR mediated recognition--or gamma delta T cells mediate antibody dependent cellular cytotoxicity (ADCC). This latter reactivity depends on Fc-receptor expression by the gamma delta T cell clones and the presence of SE specific antibodies during the assay. So far cytotoxic gamma delta T cell reactivity has only been found against the highly homologous enterotoxins SEA and SEE. Finally, HLA-class II positive gamma delta T cell clones can present SE to other SE reactive T cells but appear to be relatively resistant to T cell mediated lysis. Taken together, TCR gamma delta positive T cells are able to respond to a number of bacterial superantigens and may therefore be involved in local immune responses to such antigens. This may be especially relevant for those gamma delta T cell subpopulations that are preferentially found in the (intestinal) epithelia where exposure to bacterial superantigens is likely to occur.


In the present study, 35 Staphylococcal strain isolated from milk samples of 16 cows from eight farms of three different geographic locations in Central Java, Indonesia, and from milk samples of 19 farms from different geographic locations in Hesse, Germany, were compared pheno- and genotypically. On the basis of cultural and biochemical properties as well as by amplification of the 23S rRNA specific to Staphylococcus aureus, all isolates could be identified as S. aureus. In addition, all S. aureus isolates harboured the genes clfA and coa encoding staphylococcal clumping factor and coagulase, and the gene segments encoding the immunoglobulin G binding region and the X-region of protein A gene spa. By PCR amplification, the genes seb, seg, seh, and sei was observed for the S. aureus cultures isolated in Central Java, Indonesia and the genes sec, sed, seg, seh, sei, sej and tst for the S. aureus cultures isolated in Hesse, Germany. None of the S. aureus of both origins harbour the genes sea, see, eta and etb. All isolates were additionally positive for the genes nuc, fnbA, hla, and set1. The gene hlb was found for 6 cultures from Central Java, Indonesia and 16 cultures from Hesse, Germany. However, the gene fnbB and the gene segments cnaA and cnaB were not present among the strains isolated in Central Java, Indonesia and rare among the strains isolated in Hesse, Germany. It was of interest that most of the S. aureus isolated in
Central Java, Indonesia harboured the gene cap5 and most of the strains isolated in Hesse, Germany the gene cap8. The phenotypic and genotypic results of the present study might help to understand the distribution of prevalent S. aureus clones among bovine mastitis isolates of both countries and might help to control S. aureus infections in dairy herds.

The role of cholera toxin and heat-labile enterotoxin in the pathogenesis of diarrhoeal disease has been well documented for many years. In addition to these deleterious effects, a wealth of data is accumulating that suggests that these toxins and their subunits might be used to modulate immune responses in a variety of beneficial ways. In this regard, the toxins can boost immune responses to unrelated antigens, leading to the possibility of their use in the generation of improved vaccines to a variety of pathogens. Furthermore, recent evidence suggests that recombinant preparations of the nontoxic B subunits of the toxins have distinct immunomodulatory activities, with potential applications to the treatment of autoimmune and inflammatory diseases. This article reviews our current understanding of the mechanisms of immune modulation by these fascinating proteins.

The ability of superantigens (SAgs) to activate the immune system suggests that they may play a role in the course of autoimmune disorders. Here, Joel Schaffenbauer and colleagues review evidence from animal models of autoimmunity, as well as human data that support this hypothesis, and propose a model for SAg involvement in autoimmune disorders.

Staphylococcus aureus is a major pathogen for cattle, causing various forms of subclinical and clinical mastitis. Two groups of virulence factors (leukotoxins and superantigens) are supposed to play an important role in the initiation and/or the exacerbation of this disease. In order to detect all known and putative members of leukotoxins and SAgs (superantigens), we tested secreted factors of different S. aureus isolates in flow cytometry-based assays.Isolates were sampled from 68 cows of different farms and cultured for 24h in vitro. Supernatants were then coincubated with purified polymorphonuclear granulocytes (PMN) or combinations of blood mononuclear cells (MNC) and PMN. Viable PMN and MNC were determined by quantitative flow cytometry. In addition, we recorded the proliferation-inducing potential of isolate supernatants for bovine MNC. Based on these criteria, the supernatants of S. aureus isolates fell in three groups. The first group (n=32), termed LT-SNs (leukotoxin-containing supernatants), killed purified granulocytes (neutrophils and eosinophils) in vitro. The second group of supernatants (n=20), termed SAg-SN (superantigen-containing supernatants), induced activation and proliferation of mononuclear cells (MNC) and, only in the presence of MNC, resulted in a selective depletion of neutrophils.
after 24h in vitro. The third group of supernatants (n=16) contained neither LTs or SAgs. Functionally, SAg-SNs behaved like purified staphylococcal enterotoxin A (SEA) or SEB tested in parallel. The absence of SAg-like activity in LT-SNs was confirmed by heat treatment of LT-SNs, which destroyed the leukocytotoxic activity, but did not reveal any MNC-activating potential. This study, therefore, suggests, that pathogenic S. aureus isolates either produce leukotoxins or superantigens and that both groups of virulence factors can easily be differentiated by the functional assays described. The prevalence of leukotoxin- or superantigen-producing isolates was comparable among cattle with subclinical (LT=41%; SAg=30.8%) mastitis. The higher frequency of LT-producing isolates in cases of clinical mastitis (LT=55.2%; SAg=27.6%) was not significant. At least, these findings argue against the dominant role of superantigens or leukotoxins in S. aureus-induced bovine mastitis.


Pseudomonas aeruginosa is a potentially dangerous Gram-negative nosocomial pathogen, causing bacteremia in debilitated patients, and a prominent cause of bacterial cholangitis. Opportunistic infections with other nosocomial pathogens, e.g. Staphylococcus aureus, are common. Hence, multi-intoxication with P. aeruginosa exotoxin A (PEA) and other bacterial toxins, including endotoxin (LPS) and the superantigen S. aureus enterotoxin B (SEB), is very likely. Here we show that PEA synergistically interacted with LPS, SEB, or recombinant murine tumor necrosis factor alpha (rmuTNF) in mice, resulting in severe liver injury. Enhanced and prolonged circulation of cytokines, including TNF, which depended on the presence of T cells, was a remarkable feature of synergistic PEA/LPS- or PEA/SEB-induced hepatotoxicity. PEA/LPS-, PEA/SEB- or PEA/rmuTNF-induced liver injury was mediated by both TNF receptors (TNFRs), i.e. TNFR1 and TNFR2. In view of the fact that TNFR1, but not TNFR2, signaling is unequivocally required for host defense, our results suggest that anti-TNFR2 strategies might be beneficial to protect the liver from inflammatory damage caused by synergistic interactions of PEA with other TNF-inducing bacterial toxins.


Chemokines are mediators of innate and acquired immunity. CCL18, also designated pulmonary and activation-regulated chemokine (PARC), dendritic cell-derived CC chemokine-1 (DC-CK1), alternative macrophage activation-associated CC chemokine-1 (AMAC-1) and macrophage inflammatory protein-4 (MIP-4), was for the first time isolated from peripheral blood mononuclear cells (PBMC) and biochemically characterized. We found that CCL18/PARC protein is spontaneously secreted by PBMC and is selectively induced in PBMC by staphylococcal enterotoxins (SEA, SEB) and IL-4, but not by IFN-gamma and the CXCL8/IL-8 inducers lipopolysaccharide (LPS) or Concanavalin A. Human fibroblasts, chondrocytes and endothelial cells did not produce CCL18/PARC in response to inflammatory mediators such as measles virus, double-
stranded RNA, LPS or IL-1beta, whereas up to 150 ng/ml of CCL2/MCP-1 was induced under these conditions. In synovial fluids from septic and rheumatoid arthritis patients, fourfold-enhanced CCL18/PARC levels (150 ng/ml) were detected compared to those in crystal-induced arthritis and osteoarthritis. In septic arthritis, the synovial levels of CCL18/PARC were fivefold higher than those of CXCL8/IL-8. Immunochemistry revealed CD68(+) monocytes/macrophages as the main CCL18/PARC-producing cell type in both PBMC and arthritic synovial tissue. In addition, CD1a(+) blood dendritic cells expressed CCL18/PARC. These findings suggest that monocytes cells respond to Gram-positive bacterial infection by the production of CCL18/PARC in the synovial cavity.


PURPOSE: To investigate the presence of staphylococcal enterotoxin A (SEA) and B (SEB)-specific IgE antibodies in tears from patients with allergic conjunctival disorders.

METHODS: The study included 8 eyes of 4 patients with perennial allergic conjunctivitis (PAC), 14 eyes of 7 patients with vernal keratoconjunctivitis (VKC), 12 eyes of 6 patients with atopic keratoconjunctivitis (AKC), and 10 eyes of 10 healthy volunteers as controls. Tears were sampled by the method of the Schirmer test I. Sampled tears were eluted and SEA- and SEB-specific IgE antibodies were analyzed by the AlaSTAT-IMMULIZE method. RESULTS: SEA-specific IgE antibodies in tears were positive in 9 of 14 eyes in VKC patients and in 1 of 12 eyes in AKC patients. SEB-specific IgE antibodies in tears were positive in 7 of 14 eyes in VKC patients and in 2 of 12 eyes in AKC patients. Values for antibodies were higher in patients with severe clinical findings. However, all the cases in the normal control and the PAC groups were negative for both antibodies. CONCLUSION: Our data strongly suggested that staphylococcal enterotoxin may cause type I allergy, and may be an exacerbating factor for vernal keratoconjunctivitis and atopic keratoconjunctivitis.


Staphylococcal enterotoxins (SEs) are a family of 17 major serological types of heat-stable enterotoxins that are one of the leading causes of gastroenteritis resulting from consumption of contaminated food. SEs are considered potential bioweapons. Many Staphylococcus aureus isolates contain multiple SEs. Because of the large number of SEs, serological typing and PCR typing are laborious and time-consuming. Furthermore, serological typing may not always be practical because of antigenic similarities among enterotoxins. We report on a microarray-based one-tube assay for the simultaneous detection and identification (genetic typing) of multiple enterotoxin (ent) genes. The proposed typing method is based on PCR amplification of the target region of the ent genes with degenerate primers, followed by characterization of the PCR products by microchip hybridization with oligonucleotide probes specific for each ent gene. We verified the performance of this method by using several other techniques, including PCR amplification with gene-specific primers, followed by gel electrophoresis or microarray hybridization, and sequencing of the enterotoxin genes. The assay was evaluated by
analysis of previously characterized staphylococcal isolates containing 16 ent genes. The microarray assay revealed that some of these isolates contained additional previously undetected ent genes. The use of degenerate primers allows the simultaneous amplification and identification of as many as nine different ent genes in one S. aureus strain. The results of this study demonstrate the usefulness of the oligonucleotide microarray assay for the analysis of multitoxigenic strains, which are common among S. aureus strains, and for the analysis of microbial pathogens in general.


Recent studies have implicated various toxigenic bacteria and their toxins in the aetiology of sudden infant death syndrome (SIDS). Therefore the effect of six bacterial toxins on the cardiorespiratory system of the rabbit was studied as a model for SIDS. The toxins' effect on the heart rate, arterial blood pressure, and breathing of anaesthetized rabbits was determined and their action compared to that of endotoxin. Intravenous injection of Clostridium perfringens enterotoxin and alpha-toxin, Staphylococcus enterotoxin B, Escherichia coli heat-stable toxin (STa), Clostridium difficile toxin A and B reduced heart rate, blood pressure, respiration and increased, slowed and prolonged thorax expansion, and at higher concentrations caused sudden death without visible stress or trauma. A combination of a low concentration of enterotoxins caused a greater reduction of these activities and sudden death. These effects were generally similar to those produced by endotoxin. In non-anaesthetized rabbits, the toxins slowed metabolism until death occurred without agitation, spasms, visible distress or prolonged illness. Intestinal production of these toxins by toxigenic strains, when conditions are suitable, and their systemic absorption, could therefore cause SIDS by an endotoxin-like shock mechanism.


Targets of cyclosporine (CsA) were identified from an array of stimulated lymphocyte responses (sLR) comprising 34 stimulation conditions in whole blood from 3 normal human volunteers (NHV) containing clinically relevant CsA concentrations (0-1200 ng/ml) in vitro. In whole blood from 5 additional NHV, selected targets (intracellular interleukin-2 [IL-2], tumor-necrosis factor-alpha [TNF-alpha], and interferon-gamma [IFN-gamma]) were measured in phorbol myristate acetate (PMA)-ionomycin-stimulated T lymphocytes. Effect:concentration relationships were analyzed with E(max) pharmacodynamic (PD) equations and expressed as the concentration associated with one-half maximal inhibitory effect (EC(50)). CsA demonstrated a rich matrix of inhibitory effects on T cells (CD3(+)), B cells (CD19(+)), dendritic cells (DC) (CD11c(+)), and basophils (CD123(+)) but not on monocytes (CD14(+)) (n = 3). PD analyses suggested that the EC(50) of CsA (1) for IL-2 in CD3(+) cells in NHV (n = 8) was similar to the EC(50) demonstrated by us previously in CD4(+) cells from transplanted patients (n = 13) (EC(50) = 260 ng/ml vs. 249 ng/ml), (2) for each cytokine was different under identical stimulation conditions (TNF-alpha, 324 ng/ml; IFN-gamma,
504 ng/ml), and (3) was relatively constant for a given cytokine under different stimulation conditions (e.g., PMA-ionomycin or the staphylococcal enterotoxin B [SEB] superantigen). In conclusion, inhibition of cytokine targets by CsA is concentration dependent. Further, a given CsA concentration may produce similar inhibitory effects across different stimulation conditions. Measurement of cytokine target expression may, therefore, allow effect-controlled administration of CsA during clinical transplantation.

**Smart, et al. 2002.** Polyclonal and allergen-induced cytokine responses in children with elevated immunoglobulin E but no atopic disease. *Clin.Exp.Allergy.* 32(11): 1552-1557. BACKGROUND: Reduced Th1 and elevated Th2 cytokine responses are considered to be a principal mechanism in the generation of the inflammation leading to the manifestations of atopic disease in the skin of atopic dermatitis and in the airways of asthma. If reduced Th1 and elevated Th2 responses are principal determinants of the manifestation of atopic disease it might be expected that subjects with established disease would exhibit differences in their cytokine profiles as compared with atopic patients without clinical disease. OBJECTIVE: To determine whether asymptomatic atopic children exhibit a cytokine imbalance similar to that seen in patients with established atopic disease or if they behave like non-atopic controls. Cytokine responses in a group of children with elevated IgE but no clinical manifestations of disease, atopic children with established disease and non-atopic controls were compared. METHODS: We examined allergen-induced (house dust mite, HDM, rye grass pollen and RYE) cytokine responses in parallel with polyclonal (staphylococcal enterotoxin B, SEB) cytokine responses in a group of children with elevated serum IgE levels without current or past evidence of atopic disease (median age 6.6 years) and compared these with a non-atopic control group (median age 6.5 years) and a group of children with atopic disease (median age 6.7 years). RESULTS: Symptomatic atopic children had reduced SEB-induced IFN-gamma and increased SEB-induced IL-4 and IL-5 as compared with non-atopic controls. In contrast, SEB-induced IFN-gamma, IL-4 and IL-5 production in asymptomatic atopics was not significantly different from the non-atopic control subjects. Allergen-induced Th1 (IFN-gamma) and Th2 (IL-5 and IL-13) cytokine production was increased in both symptomatic atoples and asymptomatic atoples when compared with non-atopic controls. CONCLUSION: The defect in polyclonally induced IFN-gamma production was associated with the clinical manifestation of atopic disease but not the atopic state per se. This suggests that the global reduction in IFN-gamma is the key determinant of the development of overt atopic disease. In contrast, elevated allergen-induced Th2 cytokine responses in children related to the atopic state per se irrespective of the presence of clinical atopic disease.

**Sohn, et al. 2003.** Effect of staphylococcal enterotoxin B on specific antibody production in children with atopic dermatitis. *Allergy Asthma Proc.* 24(1): 67-71. Exotoxins secreted by Staphylococcus aureus have been identified as a possible trigger factor in atopic dermatitis (AD). We investigated the production and role of circulating antibodies, with specificity to staphylococcal enterotoxin B (SEB), in children with AD compared with those of healthy controls. The children with AD had significantly higher levels of serum SEB-specific immunoglobulin G (IgG; p = 0.0193), IgM (p = 0.011), and
IgE (p = 0.0001) than the nonatopic children. The proportions of IgG, IgM, and IgE seropositivity in children with AD were 52.5% (21/40), 62.5% (25/40), and 67.5% (27/40), respectively. The levels of SEB-specific IgE and the severity of AD (p = 0.0004) were compared, but no correlation was seen for IgG or IgM. SEB may be involved in exacerbation of AD. SEB-specific IgE may be an important index of the clinical severity of AD. The SEB-specific IgG or IgM was produced during the exposure to the SEB antigen but may not be protective against SEB in AD.


The staphylococcal enterotoxins are among the most potent T cell stimulators known. They have been shown to alter the course of disease in experimental allergic encephalomyelitis, an animal model for multiple sclerosis (MS). We have previously demonstrated that two of the staphylococcal enterotoxins, SEA and SEB, are able to reactivate paralysis in PL/J mice which had been immunized with myelin basic protein (MBP) and resolved an initial episode of paralysis. In PL/J mice, Ac1-11 is the dominant encephalitogenic determinant of MBP. We hypothesized that superantigen reactivation of experimental allergic encephalomyelitis (EAE) may result in the spreading of T cell specificities for other epitopes of MBP. PL/J mice which had resolved an initial episode of EAE were treated with SEA and developed a second episode of paralysis. At the onset of symptoms, mice were sacrificed and splenocytes were stimulated in vitro with a panel of MBP peptides. EAE reactivation by SEA resulted in the spreading of T cell specificities to residues 100 to 120 of MBP. While intramolecular spreading did occur, spreading to other antigens did not, as evidenced by the lack of response to a proteolipid protein (PLP) peptide and heat shock protein 60 (hsp 60). To further characterize the epitope MBP 100-120, PL/J mice were immunized with MBP 100-120. No initial development of disease was observed. However, administration of SEA 2 weeks after MBP 100-120 immunization resulted in the onset of paralysis. In addition to a proliferative response to MBP 100-120, these mice also exhibited a proliferative response to the flanking MBP peptides 81-100 and 120-140. Thus, SEA is able to induce intramolecular epitope spreading in PL/J mice after reactivation of EAE.


Previous work in our laboratory revealed that mice parenterally vaccinated with recombinantly attenuated staphylococcal enterotoxin (SE) or toxic shock syndrome toxin 1 develop protective antibodies against a lethal intraperitoneal (i.p.) toxin challenge. This study investigated the efficacy of nasal and oral immunizations with an SEB vaccine (SEBv) toward an i.p. or mucosal (via an aerosol) toxin challenge. Both vaccination routes, with the immunoadjuvant cholera toxin (CT), elicited comparable SEB-specific immunoglobulin A (IgA) and IgG levels in saliva. Nasal or oral inoculations also generated SEB-specific IgA, IgG, and IgM in the serum, but the nasal route yielded higher specific IgG titers. SEBv alone, when given nasally or orally, did not induce any detectable SEB-specific antibody. Mice vaccinated mucosally were protected against a
50% lethal dose of wild-type SEB given i.p. or mucosally, thus demonstrating that nasal or oral administration of this SEBv, with CT, elicits systemic and mucosal antibodies to SEB that protect against SEB-induced lethal shock.


BACKGROUND AND DESIGN: Colonization of inflammatory skin diseases with Staphylococcus aureus is a frequent phenomenon and may cause exacerbation of the skin disease. Staphylococcus aureus strains present on atopic dermatitis are capable of releasing staphylococcal enterotoxins, a group of superantigens that are very potent T-cell activators. To determine whether the superantigen staphylococcal enterotoxin B can induce inflammation when applied on the skin, staphylococcal enterotoxin B was applied with and without occlusion on the volar aspect of the skin on the forearm of 10 subjects without skin disease and six subjects with atopic dermatitis of minimal activity and no eczema on the volar aspect of the skin on their forearm. The main outcome measures were clinical rating; determination of the increase of the thickness of the skin-fold; and determination of skin blood flow. RESULTS: Clinically, staphylococcal enterotoxin B induced skin changes of erythema and induration in 10 of 10 healthy volunteer subjects and six of six subjects suffering from atopic dermatitis, while the vehicle induced clinically evident skin changes in only one of 10 healthy subjects and none of six subjects with atopic dermatitis. On day 3 after the application of an occluded patch containing 10 micrograms/cm² of staphylococcal enterotoxin B in the healthy subjects, the thickness of the skinfold increased 0.47 +/- 0.49 mm (mean +/- SD) (n = 9; P < .02) relative to the increase in the thickness of the skinfold following application of the vehicle. The Doppler laser-measured skin blood flow index had increased from 1.0 +/- 0.4 to 5.3 +/- 3.7 (mean +/- SD) (n = 10; P < .002). On day 3 after the application of occluded patches containing 10 micrograms/cm² of staphylococcal enterotoxin B in the subjects suffering from atopic dermatitis, the increase in the thickness of the skinfold increased 0.20 +/- 0.24 mm (n = 6; P, not significant) relative to the increased thickness in the skinfold following application of the vehicle. The Doppler laser-measured skin blood flow index had increased from 1.1 +/- 0.4 to 3.7 +/- 2.2 (n = 6, P, not significant). Three of six subjects suffering from atopic dermatitis experienced a flare of their disease in the elbow flexure ipsilaterally to where the staphylococcal enterotoxin B patch was applied. CONCLUSIONS: The superantigen staphylococcal enterotoxin B applied on intact skin from both normal subjects and patients with atopic dermatitis induces an inflammatory reaction. This finding suggests that superantigens released from S. aureus present on the skin in inflammatory skin diseases may exacerbate and sustain the inflammation.


Staphylococcal superantigens, including staphylococcal enterotoxin B (SEB), promote vigorous T cell-dependent Ig responses at low dose (0.01 ng/ml). In contrast, more
mitogenic high dose SEB (100 ng/ml) profoundly inhibits the Ig responses. To assess the contribution of CD8+ T cells to this inhibition, high dose SEB-dependent killing of activated B cells and down-regulation of Ig responses were determined. Rapid killing (4 h) of activated B cells was effected by high dose SEB-activated CD8+ T cells (CD8*), but not by high-dose SEB-activated CD4+ T cells (CD4*), and required the presence of high dose SEB during the cytotoxicity assay. This killing was abrogated by chelation of extracellular calcium or by treatment with concanamycin A but was only modestly affected by treatment with brefeldin A, suggesting a perforin-based pathway of killing. Despite their widely disparate abilities to rapidly kill activated B cells, CD8* and CD4* demonstrated similar quantitative abilities to effect high dose SEB-dependent down-regulation of Ig responses. Antagonist anti-CD95 mAb substantially reversed high dose SEB-dependent downregulation effected by CD8* but had no appreciable effects on high dose SEB-dependent killing of activated B cells. These observations strongly suggest that the small fraction of activated B cells that secrete Ig are selectively sensitive to CD95-based killing but resistant to CD95-independent killing. This finding may help explain why clinical autoimmunity associated with increased titers of autoantibodies is a predominant feature of defects in CD95 or CD95 ligand.

OBJECTIVES: Endovascular repair of abdominal aortic aneurysms (E-AAA) has in recent years developed as an alternative to the conventional open repair (C-AAA). Adverse outcomes following the open approach may relate to immune cell activation and the systemic inflammatory response syndrome (SIRS) and organ failure but the benefits in this respect of the endovascular approach are unclear. This study evaluated this question and focused on T-cell activation and function. DESIGN: prospective clinical study. MATERIALS: twenty patients undergoing abdominal aortic aneurysm repair (12 C-AAA and 8 E-AAA). METHODS: peripheral T-cell expression of surface markers CD69, CD62L and CD25 in vivo and Interleukin 2 (IL-2) and Interleukin-10 (IL-10) responses to the superantigen staphylococcal enterotoxin B (SEB) in vitro were measured preoperatively, 24 h and 1 week postoperatively. RESULTS: There was no significant increase (p=0.23) in the incidence of SIRS in the open compared with the endovascular group. Enhanced T cell activation occurred following C-AAA and this was associated with significantly greater IL-2 production in response to SEB, with no change in IL-10 production. CONCLUSIONS: E-AAA attenuates proinflammatory T-cell changes compared with C-AAA repair. A reduction in T-cell activation and impaired responsiveness to superantigen suggests that the immunological sequelae of the endovascular approach to aneurysm repair is more favourable than after the open approach with potentially less risk of adverse outcomes. Proof of this thesis will require a larger prospective study.

1. Staphylococcal enterotoxine B (SEB; superantigen) accelerated the onset of arthritis in mice preimmunized with type II collagen (SEB-potentiated collagen-induced arthritis).
Cyclosporin A and FK-506 inhibited the induction and development of clinical signs and histopathological changes of SEB-potentiated collagen-induced arthritis in mice. 2. Simultaneously, both cyclosporin A and FK-506 inhibited the development of humoral and cellular immunity to type II collagen. 3. The expression of IL-2 receptor (CD25) by SEB on splenocyte T cells from collagen-preimmunized mice was inhibited by both agents in ex vivo experimentation.


Activated T lymphocytes appear to be responsible for liver damage in chronic active hepatitis and autoimmune liver disease. We described three experimental mouse models of T cell-dependent liver injury. D-galactosamine (GalN)-sensitized mice challenged with either T cell activating anti-CD3 monoclonal antibody (mAb) or with the superantigen staphylococcal enterotoxin B (SEB) developed severe liver injury characterized by internucleosomal DNA fragmentation as well as by histological hallmarks of hepatocyte apoptosis, both preceding the increase of plasma transaminases. Administration of the T cell mitogen concanavalin A (Con A) to unsensitized mice also resulted in hepatic apoptosis and the ensuing necrosis. Anti-CD3 mAb as well as SEB or Con A induced the release of systemic tumor necrosis factor (TNF), interferon gamma (IFN gamma), and various other cytokines. Passive immunization against TNF or pretreatment with immunosuppressive drugs such as cyclosporin A, FK 506 or dexamethasone protected mice from liver injury. T lymphocytes were identified as effector cells of Con A in vivo i) by proof of resistance of athymic nude mice against Con A and ii) by restoration of susceptibility in nude by lymphocyte transfer from control mice. Moreover, antibody-dependent depletion of CD4+ T cells fully protected against Con A, whereas depletion of CD8+ T cells failed to prevent liver injury. These results indicated that cytokines released following T helper cell activation rather than cytotoxic T cells mediated liver injury. We recently found that IFN gamma is also a critical mediator of Con A-induced hepatic damage. In conclusion, these T cell-dependent models of inflammatory liver injury allow the investigation of basic principles of hepatic disorders associated with T cell activation and infiltration as well as pharmacological in vivo studies for the development of hepatoprotective drugs.


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Microbial toxins as well as certain drugs and xenobiotics exert their toxic potential towards mammalian organisms by either activation or suppression of the immune system. We have investigated the immune stimulatory effect of either the bacterial superantigen staphylococcal enterotoxin B (SEB) or of the T cell activating anti-CD3 monoclonal antibody (alpha CD3-mAb) or of the T cell mitogenic plant lectin concanavalin A (Con A) in murine in vivo and in vitro systems. Any of these agents evoked a strong cytokine response in vitro and in vivo. Tumor necrosis factor-alpha (TNF) was identified as a common cytotoxic mediator which induced hepatocyte apoptosis as characterized by histological examination and internucleosomal DNA fragmentation, that preceded the release of liver specific enzymes into plasma and the histological appearance of necrosis. These mechanisms of acute liver failure observed under experimental conditions are discussed to account also for liver injury during acute episodes of autoimmune or viral hepatitis or rejection of liver grafts.


The seleno-organic drug ebselen (2-phenyl-1, 2-benzoisoselenazol-3(2H)-one) has glutathione peroxidase-like activity, and inhibits lipoxygenases, oxidative burst of leukocytes, nitric oxide synthases, protein kinases and leukocyte migration. This study elaborates in vivo in mice hitherto unknown immunopharmacological properties of ebselen. The compound was comparatively investigated in two different T cell-dependent hepatic hyperinflammation models and in two alternative models of receptor-activated liver apoptosis. Mice orally pretreated with ebselen were dose-dependently protected from concanavalin A (ConA)-induced liver injury. In livers from ebselen-pretreated mice exposed to ConA, the nuclear antiapoptotic transcription factor NFkappaB was upregulated. The release of the proinflammatory cytokine tumor necrosis factor-alpha (TNF) was downregulated, while the circulating amount of the anti-inflammatory cytokine interleukin-10 (IL-10) was increased. Ebselen protected also from liver injury induced by the superantigen staphylococcal enterotoxin B in galactosamine (GalN)-sensitized mice.
Furthermore, ebselen protected the liver and enhanced circulating IL-10 in GalN-sensitized mice treated with recombinant TNF, i.e., the common distal mediator of ConA and SEB-induced hepatotoxicity. The activation of apoptosis-executing proteases, i.e., caspases, was blocked in livers of ebselen-treated mice following TNF receptor, but not following CD95 receptor activation. We propose a novel mechanism for the immunomodulatory properties of the drug and suggest that it might be useful in the therapy of T cell-mediated inflammatory disorders.


Hypersensitivity pneumonitis (HP) and sarcoidosis are interstitial lung disorders (ILD) characterized by a lymphocytic alveolitis that, in the active phase of the disease, is sustained by different T-cell subsets, i.e., CD8+ cells in HP and CD4+ lymphocytes in sarcoid patients. To address the question of whether a bias in T-cell selection occurs in the lung of patients with HP and sarcoidosis, we analyzed the T-cell receptor beta chain variable region (TCR-Vbeta) repertoire by flow cytometry and polymerase chain reaction (PCR) analyses in blood and lung lymphocytes of 14 HP and 25 sarcoid patients. To verify whether these cells can be activated in vitro through the TCR, blood and lung lymphocytes were also assessed for their responsiveness to different superantigenic stimuli represented by staphylococcal enterotoxins, including SEA, SEB, SEC1, SEC2, SED, and SEE. Flow cytometry and PCR analyses demonstrated an overexpression of cells bearing Vbeta2, Vbeta3, Vbeta5, Vbeta6, and Vbeta8 gene segments in the lung of HP patients as compared with the peripheral blood. In sarcoid patients cells bearing Vbeta2, Vbeta5, and Vbeta6 gene segments were overrepresented in the lung rather than in the blood. Both in HP and sarcoid patients almost all T cells bearing the dominant Vbeta segment belonged to the T-cell subset that sustains the alveolitis, i.e., CD8 in HP patients and CD4 in sarcoid subjects. Follow-up studies demonstrated that the recovery of the alveolitis was characterized by the disappearance of cells bearing a limited T-cell repertoire. Interestingly, T-lymphocyte response to different superantigens demonstrated that the proliferation elicited by different staphylococcal toxins was more pronounced in the lung than in the blood. Taken together, our findings indicate a compartmentalization of cells bearing discrete Vbeta gene products in the pulmonary microenvironment and suggest that the expansion of specific Vbeta region subsets occurring in the lung might result from triggering by a specific antigen. In fact, the removal from exposure in HP patients or specific treatment in sarcoidosis resulted in the decrease of the overrepresented cell population accounting for the lymphocytic alveolitis.


Cholera toxin (Ctx) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here we describe findings which demonstrate that contrary to the established dogma the non-toxic B-subunit of Etx (EtxB) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.


The staphylococcal enterotoxins (SEs) are capable of causing both food poisoning and a toxic shock-like illness in man. In addition, SEs are known to act as superantigens, stimulating T-cells according to their T-cell receptor Vbeta type. Relatively little is known of their antigenic determinants and how these may relate to the structure and function of the toxins. As a step in the study of these relationships, the entire molecule of SEB was synthesized in duplicate as a series of octapeptides overlapping by seven residues. This series thus represented all the potential linear epitopes of eight residues or less. The reactivity of the octapeptide series with antisera raised to purified SEB and to formaldehyde-inactivated SEB has been used to locate several antigenic sites on native SEB and to identify antigenic differences in the toxoid. Three antigenic peptides identified from the antigenic profile were synthesized and characterized. These represented amino acids 21-32, 93-107 and 202-217 of SEB. None of these peptides affected SEB-induced T-cell proliferation. However, the occurrence or absence of cross-reactivity of these peptides with antibodies to native SEB corresponds to the degree of exposure and/or the rigidity of these regions within SEB.


OBJECTIVE--To observe the influence of T cell subset changes on the development of experimental arthritis, by using the bacterial superantigen staphylococcal enterotoxin B
(SEB) to modulate the T cell repertoire during the arthritogenic response to type II collagen (CII) in vivo. METHODS--DBA/1 mice were injected with SEB before immunisation with CII, and assessed for the development of collagen induced arthritis (CIA) and an immune response to CII. Mice with established arthritis were also treated therapeutically with SEB. Flow cytometry was used to evaluate the effect of the therapy on T cell subsets and T cell receptor (TCR) V beta expression. RESULTS--Mice injected with SEB developed arthritis significantly faster than saline treated control animals, and developed more severe clinical features. Mice treated with SEB after the onset of CIA were also observed to progress more rapidly to a severe arthritis than mice treated with saline alone. The level of anti-CII antibody was not affected by SEB injection. Flow cytometric analysis of TCR expression in mice 21 days after injection of CII showed decreased expression of V beta 6 and V beta 8 cells in SEB treated mice, compared with collagen immunised control mice. Injection of SEB alone caused a decrease in V beta 8, but not V beta 6 T cells compared with the values in normal DBA/1 mice. No significant variations in the T cell repertoire were detected 70 days after CII immunisation. CONCLUSIONS--Treatment with the bacterial enterotoxin SEB before the induction of arthritis did not suppress the immunological or arthritogenic response to CII in DBA/1 mice, despite the modulation of the V beta 8 T cell subset. Treatment of mice with established arthritis using SEB provoked a more severe disease course.


It has been recently hypothesized that superantigens play a precipitating or aggravating role in psoriasis. Aside from streptococcal infection, Staphylococcus aureus can be sometimes detected in the tonsils of patients with psoriasis arthropathy (PA), although its significance in the pathogenesis of PA is still unknown. These focal infections are thought to be a possible triggering factor of the arthralgia, as well as the cutaneous manifestations, in PA. In this study, we have investigated the response of peripheral blood mononuclear cells (PBMC) from patients with PA to staphylococcal superantigens and analyzed its association with clinical and laboratory findings. 3H-TdR uptake by PBMC was examined after 7 days' culture with concanavalin A (Con A), staphylococcal enterotoxin A (SEA), SEB and SEC1. Results showed that there was no significant difference in either the unstimulated or Con A-stimulated PBMC response between psoriasis vulgaris patients (PASI score < 10) (n = 15), PA patients (n = 11) and normal controls (n = 19). Among 11 PA patients, 8 patients responded most intensely to SEB, while 2 patients showed the strongest response to SEA, and another responded mainly to SEC1. The PBMC response against SEB in patients with PA (38,715 719 dpm, stimulation index (SI); 50.2 41.4) (mean SD) was significantly higher than that in normal controls (23,708 466 dpm, SI; 30.9 23.8) (p < 0.05), however, the difference between that of patients with PA and psoriasis vulgaris (33,428 467 dpm, SI; 42.8 30.6) did not reach significance. In addition, PBMC from psoriatic patients with a short episode of severe, disabling lumbago, which occurred following sudden onset throat soreness, showed a stronger response against SEB (SI; 73.7 39.7), as compared with that of PA patients without such an episode (SI; 42.6 18.1). However this difference did not reach
significance. Several immune abnormalities, including positive antinuclear antibodies or rheumatoid factor were observed mainly in the group experiencing such an episode of severe lumbago. Reverse transcriptase-polymerase chain reaction (RT-PCR) demonstrated that predominant expression of the T cell receptor (TCR) Vbeta 17 was commonly detected in both synovial tissues and paired peripheral bloods in two cases examined. In one case, Vbeta 12 was preferentially expressed, and in another case, Vbeta 10, 15 and 19 were also strongly expressed in the infiltrating lymphocytes in the synovial tissues. Our data raised the possibility that staphylococcal superantigens may also play an exacerbating role in PA.


It has been recently hypothesized that superantigens, which stimulate T cells expressing particular T cell receptor Vbeta chain gene segments, play a precipitating or aggravating role in psoriasis. In this study, we investigated the peripheral blood mononuclear cell (PBMC) response of patients with psoriasis vulgaris to staphylococcal superantigens (staphylococcal enterotoxin A (SEA), SEB, and SEC1) and its relationship to clinical and laboratory findings. Cytokine secretion was assessed by ELISA in the supernatants of the cultured PBMCs stimulated with SEB. Results of 3H-TdR uptake showed that the PBMCs' response against SEB in patients with psoriasis vulgaris (34,468 +/- 6,455) (mean DPM SD) was significantly higher than that of normal subjects (22,756 +/- 5,780) (p < 0.005). The stimulation index (SI) of patients with psoriasis vulgaris (n = 37) (63.9 +/- 55) was significantly higher than that of normal subjects (n = 24) (26.0 +/- 23) (p < 0.005) and patients with atopic dermatitis (n = 10) (40.7 +/- 30) (p < 0.05). Similar results were obtained in response to SEA and SEC1. SI weakly correlated with the psoriasis area and severity index (PASI) score (r = 0.62) and the serum interleukin-6 (IL-6) concentration (r = 0.45). IL-2 and tumor necrosis factor (TNF-alpha) were secreted at a significantly increased level by PBMCs from psoriatic patients on incubation with SEB, after a 3 day culture period. A higher level of IL-6 was released by PBMCs stimulated with SEB in psoriatic patients than normal controls, however, the difference was not significant. These results raise the possibility that monocytes, as well as T cells, are markedly activated by staphylococcal superantigen in patients with psoriasis vulgaris, which may play a role in the triggering or aggravating of psoriasis mediated by secreted cytokines.


Autoreactive T cells specific for myelin basic protein (MBP) are part of the normal T cell repertoire and are present both in patients with multiple sclerosis (MS) and healthy individuals. There is evidence suggesting in vivo activation and persistent clonal expansion of MBP-reactive T cells in MS. This study was undertaken to investigate the potential role of bacterial superantigens (SA) in the activation of MBP-reactive T cells. Twenty-seven MBP-reactive T cell clones generated from 10 MS patients and one normal individual were examined for reactivity to SA, in association with their T cell receptor V beta gene usage. The majority of the clones responded to at least one of the SA tested,
staphylococcal enterotoxins (SEA and SEB) and toxic shock syndrome toxin-1 (TSST-1). The clones reactive to SEA and SEB expressed various V beta genes while T cell reactivity to TSST-1 correlated with the V beta 2 expression. Furthermore, circulating MBP-reactive T cells could be expanded from lymphocyte cultures primarily exposed to respective SA in more than 50% of MS patients and normal individuals tested. However, activation and expansion of circulating MBP-reactive T cells by SA was not directly associated with the disease. This study lends support to the potential role of SA in the activation of MBP-reactive T cells and suggests that an altered regulatory mechanism may account for further expansion and persistence of MBP-reactive T cells in MS.