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Staphylococcal superantigens and toxins are detectable in the serum of adult burn patients☆,☆☆

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ABSTRACT

Bacterial infection in burn patients is still a devastating contributor to morbidity and mortality. Little is known regarding the presence of staphylococcal toxins in the burn-injured patient. The aim of this study was to characterize the prevalence of several of these toxins and their relationship to clinical metrics and mortality in burn patients. Levels of exotoxins staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B, toxic shock syndrome toxin 1 (TSST-1), and α-hemolysin were assayed from the serum of 207 adult burn patients aged 16–92 years. Clinical, demographic, and microbiological data from these patients were then compared to toxin levels. Staphylococcal exotoxins α-hemolysin and SEA were present in 45% and 25% of the population, respectively. Bacterial cultures concomitantly showed a high prevalence of *Staphylococcus aureus* in 48% of patients, of which 59% were methicillin resistant. Several metrics may be predictive of high toxin concentrations of α-hemolysin and TSST-1 and SEA including burn size, length of stay, and bacteremia. Mortality associations indicated that burn size, bacteremia, age, and the presence of α-hemolysin and SEA may be predictors of mortality. A high prevalence of staphylococcal toxin α-hemolysin and superantigens TSST-1 and SEA can be found in the circulation of the adult burn population. The presence of these toxins may contribute to the morbidity and mortality of the burn patient.

1. Introduction

The local and systemic effects of bacterial virulence factors on the pathophysiology of thermal injury have yet to be well defined. Burn patients present several unique challenges with regard to immune defense, infection control, and susceptibility to multiple episodes of transient bacteremia and sepsis. Burn-injured skin remains as an open wound for extended periods of time, and patients have a higher incidence of indwelling catheter- and ventilator-associated infections. Immune dysfunction has been described to result from thermal injury, including: impaired cytotoxic T lymphocyte response, arrested myeloid maturation, impaired neutrophil response, and decreased macrophage production (Avni et al., 2010; Gamelli et al., 2000; Xiu and Jeschke, 2013).

The most frequent organisms associated with burn-injured patients are *Pseudomonas* and *Staphylococci* spp. (75% of cases) (Bi et al., 2009). *Staphylococcus aureus* produces an array of virulence factors that contribute to the organism's invasiveness and histotoxicity. Some of these products help the organism evade the host's immune system, while other factors assist in the destruction of skin and connective tissue in the process of creating a wound infection. A third group of products are exogenously produced and termed exotoxins. These proteins are most known for their ability to induce shock; however, specific cellular and molecular interactions have been hypothesized. Staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1) are members of this family (Becker et al., 2001; da Cunha et al., 2007; Veras et al., 2008). These pyrogenic exotoxins have the characteristic ability to simultaneously bind both human leukocyte antigen (HLA-DR) (or DQ) and the T-cell receptor, achieving T lymphocyte stimulation levels of up to 20%, far greater than that of conventional antigens (Johnson et al., 1991a; Johnson et al., 1991b; Johnson et al., 1992; Marrack et al., 1990; Marrack and Kappler, 1990). Much of the pathology encountered subsequent to exposure of SE has been attributed to this superantigenic interaction, which causes elevated serum cytokine levels, specifically tumor necrosis factor alpha and interleukin-2 (Jett et al., 2002). Interestingly, there have been several reports showing that SEs are capable of inducing tissue-specific interactions without the presence of lymphocytes, antigen presenting cells, or their cytokines. Such tissues include pulmonary endothelium, renal proximal tubule epithelial cells, and colonic epithelium (Jett et al., 2002; Campbell et al., 1997; Chatterjee and Jett, 1992; Shupp et al., 2002; van Gessel et al., 2004).

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Most recently in an animal model of *S. aureus*–infected burn wounds, toxin was identified in renal tissue. Notably, the animals in this report never exhibited bacteremia (Mino et al., 2013).

SE exposure in the critically ill may have a devastating result as many of the systems affected during enterotoxicosis are pivotal in sustaining homeostasis. Currently, little is known about the presence of superantigens in critically ill patients, except that, in 2004, Azuma et al. (2004) demonstrated that SE was detectable in a sample intensive care unit (ICU) population; however, only 13% were thermally injured. In addition, some in vivo research has been completed in animals showing the possible contributions of superantigen exposure to burn sepsis and shock. These studies point to the immune dysfunction and suggest negative implications of enterotoxin exposure in parallel to burn injury (Li et al., 2003).

While there is evidence to suggest that these toxins are present in ICU patients, not all burn patients suffer septic complications, a yet unexplained enigma warranting a more extensive examination of the thermally injured population. In this work, blood samples from burn patients at a large urban verified burn center were examined for presence of α-hemolysin and superantigens TSST-1, staphylococcal enterotoxin A (SEA), and staphylococcal enterotoxin B (SEB). Toxin positivity was then correlated with clinical parameters, and outcome associations were assessed.

2. Methods

2.1. Study design and participants

The Institutional Review Board of MedStar Health Research Institute approved this study. The patient population consisted of 207 consecutively admitted burn-injured adult patients presenting to an urban verified burn center between March 2009 and April 2010. Excess sera from patients' labs being stored at 4 °C (maximum 48 hours) were obtained and aliquoted for storage at −80 °C until assays were completed.

2.2. Toxin protein quantification

Toxin concentrations of TSST-1, SEA, SEB, and α-hemolysin were determined by modified sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (Mino et al., 2013) using commercially available protein as standards (Toxin Technology, Inc., Sarasota, FL, USA). Patient samples were assayed in duplicate with many in triplicate. To develop the standard curve, the optical density of wells in duplicate was quantified to determine the amount of antibody-bound toxin. The error of the standard curve was analyzed using the calibration curve error equation, and toxin-positive samples were characterized as having a concentration greater than the range of individual SE.

High levels of toxins were established as: 50 ng/mL for TSST-1, 88.3 ng/mL for both SEA and SEB, and 1.4 μg/mL for α-hemolysin based on historic data (Gill, 1982; Goshi et al., 1963; Hodoval et al., 1968; Lominski et al., 1963; Pettit et al., 1977; Reeves et al., 1986; Savransky et al., 2003; Stiles et al., 1993; Ulrich et al., 1997; Watanabe and Kato, 1974; Wiseman, 1975). Since the physiologic implications of historically high levels of toxin are not known in this patient population, all samples below this threshold were considered low values.

2.3. Patient data collection

Demographics, clinical microbiology, and outcomes were collected retrospectively from medical record reviews of all patients. Culture data that were abstracted from the medical records included the following sites (blood, bronchial, burn wound, catheter tip, drainage fluid, nasal, sputum, and urine). All cultures and sensitivities recorded from the patients' clinical records were generated from the hospitals clinical laboratory, in accordance with the standard methodologies.

2.4. Statistical analysis

All clinical data were abstracted from charts and entered into an Access (Microsoft Corp., Redmond, WA, USA) relational database along with toxin ELISA results using SAS 9.4® (Cary, NC, USA). Predictive relationships of toxin levels and mortality to each metric of culture results, burn size, and length of stay were determined by ordered logistic regression controlling for various factors (Tables 3 and 4).

3. Results

3.1. Study cohort characteristics

Of the 207 subjects, 62% were male and 40% had flame burns. Other burn injuries included flame, scald, grease, contact, electrical, and others. The age range was 16–92 years, mean burn size was 9.5%, and mean length of stay was 31 days. Twelve fatalities (5.8%) were included in this study.

3.2. Exotoxin prevalence

Staphylococcal exotoxin levels of TSST-1, SEA, SEB, and α-hemolysin from 1566 serum samples are shown in Table 1. Present in 672 (42.9%) samples, α-hemolysin was the most prevalent of toxins, followed by the superantigen SEA at a frequency of 210 (13.4%) samples. Distribution of these toxins among the population (Table 1) follows a similar trend, as 94 patients (45.4%) were positive for α-hemolysin, and 51 (24.6%) for SEA at least once during their stay.

3.3. Bacterial burden

Cultures from all body sources were collected clinically for 91 of the 207 patients with microorganism frequency in Fig. 1. Of these 91 patients, 50 cultured *Staphylococcus* spp. *S. aureus* was present in 44 (88%) of cases, and 26 (52%) were methicillin resistant (Table 2). Cultures specifically of blood samples were collected on 66 patients, 26 of which had bacterial isolates, and of these, 19 (73%) were *Staphylococcus* spp. Staphylococcal bacteremia cases consisted of 5 (26%) coagulase-negative, 9 (47%) coagulase-positive, and 5 (26%) cases of infection by both species. Coagulase-positive cases were

Table 1
Toxicology characteristics among the burn population.

	Toxin concentration (ng/mL)			Toxin level, no. (% of patients)		
	Positive samples[a] (no.)	Mean (SD)	Range	Negative	Low	High
TSST-1	77	29.4 (29.9)	3.6–167.8	179 (86.47%)	25 (12.08%)	3 (1.45%)
SEA	210	65.9 (144.8)	3.1–1310.3	156 (75.36%)	42 (20.29%)	9 (4.35%)
SEB	28	38.1 (25.3)	12.7–112.5	199 (96.14%)	6 (2.90%)	2 (0.97%)
α-Hemolysin	672	539.9 (901.1)	25.5–6430.1	113 (54.59%)	85 (41.06%)	9 (4.35%)

[a] Positive samples reflect an uneven distribution of 1566 results per toxin among 207 patients.