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Systemic cytokine and chemokine responses in immunized mice challenged with staphylococcal enterotoxin B

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ABSTRACT

The cytokine storm induced by staphylococcal enterotoxin B (SEB) describes the rapid and dramatic induction of mediators which are likely responsible for the toxin's deleterious effects. However despite the use of numerous animal models for investigating SEB related illness in humans, mechanisms of toxicity and correlates of protection remain unclear. In the present study, we used an LPS-potentiated model of SEB lethality to investigate the toxin-induced cytokine and chemokine responses in untreated and immunized mice. Of 30 separate mediators analyzed, serum levels for 28 or 27 of these cytokines and chemokines were elevated following administration of dosages of 3 or 30 LD_{50} of native SEB, respectively. Mice immunized with a non-toxic SEB vaccine candidate expressed in either *E. coli* or transgenic soy expression systems were protected from lethality when challenged with potentiated SEB. The majority of SEB-induced cytokines and chemokines (21 of 28 or 23 of 27 following challenge with dosages of 3 or 30 LD_{50} of native SEB, respectively) were significantly decreased in mice immunized with an SEB vaccine candidate when compared to control animals. Together, these studies provide the most comprehensive evaluation of the cytokine storm induced in this LPS-potentiated model of SEB lethality to date. As with other animal models, the identification of those mediators which are necessary and sufficient for SEB-induced toxicity remains unclear.

1. Introduction

Animal models to evaluate human vaccine candidates have been widely utilized in research and development efforts (Gerdts et al., 2007) and are required for regulatory agency approvals (Riese et al., 2015). Unfortunately, animal models used for pre-clinical vaccine efficacy studies have some significant limitations (Centlivre and Combadiere, 2015). Rodent models are relatively inexpensive, and there are many reagents available to characterize their immune responses (Riese et al., 2015). The ability to characterize the magnitude of an immunoglobulin or cell mediated response following vaccination of rodents is routine (Schunk and Macallum, 2005). However it has often been difficult to determine whether a particular antibody level, or the expansion of a particular antigen-specific lymphocyte population, represents a correlate of protection in rodents that will translate to vaccine efficacy in humans (Thakur et al., 2012). In an effort to engineer relevant responses in mice, methods such as the xenotransplantation of human immune cells or tissues into immunodeficient mouse strains have been developed (Centlivre and Combadiere, 2015). Non-rodent vaccination models more closely mimic human immune responses, and the relevance of these larger animal models has been touted (Gerdts et al., 2015). In particular, non-human primate models likely provide the most comparable immunity following vaccination (Rivera-Hernandez et al., 2014). However the expense and limitations, including ethical considerations, for justifying significant numbers of primates for post-immunization challenge studies often restrict their routine use

Abbreviations: mSEB, a recombinant form of staphylococcal enterotoxin B (SEB) containing the three amino acid mutations, L45R, Y89A, Y94A; KLH, keyhole limpet hemocyanin.

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(Rivera-Hernandez et al., 2014).

A variety of animal models have been used to investigate the efficacy of vaccine candidates targeting staphylococcal enterotoxin B (SEB) (Brosnahan, 2016; Fries and Varshney, 2013). The relative insensitivity of normal mice to this toxin has resulted in potentiation of its effects by co-administration of agents such as LPS (Stiles et al., 1993) or D-galactosamine (Miethke et al., 1992) to produce lethality. Alternatively, transgenic mice which express human major histocompatibility class II molecules have been engineered resulting in a more robust response to SEB (Faulkner et al., 2005; Rajagopalan et al., 2009). Larger animal models respond systemically to SEB as a superantigen (Krakauer et al., 2016), including swine (Bost et al., 2016; Hudson et al., 2013) and non-human primates (He et al., 2014; Komisar et al., 2001; Weng et al., 1997). These models have also been used to suggest efficacy of various SEB vaccination formulations (Boles et al., 2003; Hudson et al., 2013; Tseng et al., 1995). Despite such a diversity of studies using a variety of animal models, the immune parameters central for mediating SEB toxicity in humans remain unclear (Fries and Varshney, 2013; Krakauer et al., 2016). An SEB-induced cytokine storm is universally described as being deleterious (Tisoncik et al., 2012). However, those secreted factors which directly initiate this cascade, or the magnitude necessary to mediate SEB's noxious effects, remain poorly defined (Fries and Varshney, 2013; Krakauer et al., 2016).

In the present study, we utilized an LPS-potentiated model of SEB lethality to investigate the toxin-induced cytokine and chemokine responses in mice. While previous studies using this model have focused on systemic production of a few mediators (Stiles et al., 1993, 2001; Ulrich et al., 1998), we were able to provide a more comprehensive characterization using a fluorescent multiplex assay. In addition, mice immunized with a SEB vaccine candidate produced using two different recombinant protein expression platforms (Hudson et al., 2013, 2014) were challenged with increasing doses of native toxin to investigate the ability of vaccination to reduce the lethal, SEB-induced cytokine storm. For those cytokines and chemokines which were induced by native SEB challenge, vaccinated mice demonstrated significantly reduced serum levels in most of these toxin-induced mediators. The relative magnitude of this reduction was variable depending on the particular endogenous mediator being quantified. Unfortunately, the large number of SEB-induced cytokines and chemokines, and their magnitude of expression, provided little insight as to which factors were required or were necessary for the initiation or lethality of this toxin-induced cascade.

2. Methods

2.1. Expression and purification of a non-toxic SEB vaccine candidate in E. coli

A non-toxic form of SEB containing the three amino acid mutations, L45R, Y89A, Y94A, was expressed in *E. coli* (Hudson et al., 2013, 2014). This vaccine candidate, designated *E. coli*-mSEB, was purified as previously described (Hudson et al., 2013, 2014) and used in these studies. Briefly, the mSEB open reading frame containing the amino acid changes, L45R, Y89A, Y94A, was cloned into a pET expression vector and transformed into *E. coli*. Cultures containing the expression plasmid were grown to stationary phase in terrific broth at 37 °C for 16 h, and cells were harvested by centrifugation. The cell paste was resuspended in 50 mM phosphate buffer (pH 6.3) and sonicated on ice. Insoluble material was removed by centrifugation, and nickel resin was used to bind protein from the soluble fraction. Recombinant protein was eluted with 50 mM phosphate buffer containing 400 mM imizazole. Eluted *E. coli*-mSEB was quantified using the Bradford reagent (with BSA as a standard) and purity was determined by Coomassie-blue staining of SDS-PAGE gels.

2.2. Expression and purification of a non-toxic SEB vaccine candidate in transgenic soybean seeds

A non-toxic form of SEB containing the three amino acid mutations, L45R, Y89A, Y94A, was expressed in transgenic soybean seed as previously described and was designated soy-mSEB (Hudson et al., 2013, 2014). Briefly, *agrobacterium*-mediated soybean transformations were performed and transgenic events were taken to maturity. T1 seeds were collected and genomic and protein assays were conducted to confirm DNA integration and protein expression. Based on results from these assays, select seeds were germinated and propagated over several generations.

Soy-mSEB was isolated as previously described (Hudson et al., 2013, 2014). Briefly, transgenic seeds were ground to a fine powder, and protein extracted by sonication in 50 mM Tris-HCl (pH 7.8). The sonicated protein mixture was clarified by centrifugation, and the pH was lowered to 4.5 to precipitate acidic proteins. Insoluble material was removed by centrifugation, and the soluble extract was sequentially passed over DEAE cellulose, and then CM cellulose, columns to purify soy-mSEB. Purified mSEB was quantified using the Bradford reagent (with BSA as a standard) and purity was determined by Coomassie-blue staining of SDS-PAGE gels.

2.3. Immunization of BALB/c mice with E. Coli-mSEB or soy-mSEB

These procedures for immunizing mice were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Charlotte. Groups of 4 week old BALC/c mice (N = 7) were immunized and boosted intramuscularly with 50 μg of purified *E. coli*-mSEB or soy-mSEB emulsified in Freund's adjuvant on days 0, 15, and 29, respectively. Groups of control animals (N = 6) received intramuscular injections of 50 μg of an irrelevant antigen (keyhole limpet hemocyanin, KLH) emulsified in Freund's adjuvant on the same days. Blood was taken from individual animals in each group via the saphenous vein prior to each immunization (days 0, 15, and 29), and also at day 58. Sera was isolated by centrifugation and stored at −80 °C until used for anti-SEB antibody quantification.

2.4. ELISAs to quantify the developing anti-SEB serum antibody response and endpoint IgG anti-SEB titers

To quantify the anti-SEB serum antibody response following immunizations, ELISA plates (Corning, Corning, NY) were coated with 100 ng/well of native SEB (Toxin Technologies, Inc., Sarasota, FL) in 100 μL of carbonate buffer overnight at 4 °C. Wells were washed, and blocked with 3% BSA in PBS for 1 h at room temperature. A 1:3000 dilution of sera from each mouse was added to wells and incubated overnight at 4 °C. Wells were then washed and a horseradish peroxidase conjugated anti-mouse IgG antibody (Southern Biotechnology, Birmingham, AL) added at a dilution of 1:2500 in 1% BSA for 1 h at room temperature. After washing, TMB substrate (BioFX, Owings Mills, MD) was added. Enzymatic reactions were stopped by addition of 1 M sulfuric acid and absorbance at 450 nm recorded.

To determine anti-SEB antibody endpoint titers (day 58), a similar ELISA procedure was used. For this ELISA, serum from each mouse was serially diluted as indicated before performing the analysis.