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Natural indoles, indole-3-carbinol and 3,3′-diindolymethane, inhibit T cell activation by staphylococcal enterotoxin B through epigenetic regulation involving HDAC expression

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ABSTRACT

Staphylococcal enterotoxin B (SEB) is a potent exotoxin produced by the *Staphylococcus aureus*. This toxin is classified as a superantigen because of its ability to directly bind with MHC-II class molecules followed by activation of a large proportion of T cells bearing specific Vβ-T cell receptors. Commonly associated with classic food poisoning, SEB has also been shown to induce toxic shock syndrome, and is also considered to be a potential biological warfare agent because it is easily aerosolized. In the present study, we assessed the ability of indole-3-carbinol (I3C) and one of its byproducts, 3,3′-diindolylmethane (DIM), found in cruciferous vegetables, to counteract the effects of SEB-induced activation of T cells in mice. Both I3C and DIM were found to decrease the activation, proliferation, and cytokine production by SEB-activated Vβ8$^+$ T cells in vitro and in vivo. Interestingly, inhibitors of histone deacetylase class I (HDAC-I), but not class II (HDAC-II), showed significant decrease in SEB-induced T cell activation and cytokine production, thereby suggesting that epigenetic modulation plays a critical role in the regulation of SEB-induced inflammation. In addition, I3C and DIM caused a decrease in HDAC-I but not HDAC-II in SEB-activated T cells, thereby suggesting that I3C and DIM may inhibit SEB-mediated T cell activation by acting as HDAC-I inhibitors. These studies not only suggest for the first time that plant-derived indoles are potent suppressors of SEB-induced T cell activation and cytokine storm but also that they may mediate these effects by acting as HDAC inhibitors.

Introduction

Staphylococcal enterotoxin B (SEB) is a 28-kDa protein belonging to a family of exotoxins secreted by the bacterium *Staphylococcus aureus* (*S. aureus*), a ubiquitous Gram-positive coccus that has been found to colonize both human and domestic animals as a common opportunistic pathogen. It is estimated that *S. aureus* can be found in 20% of the general population, with 60% of those being intermittent carriers, and has become a major cause of nosocomial infections and community-acquired diseases (Pinchuk et al., 2010). Growing worldwide concern has emerged with the discovery that many incidences of these nosocomial infections involve the methicillin-resistant (MRSA) strain of *S. aureus*, with a majority of this particularly dangerous antibiotic-resistant strain producing toxins, such as SEB (Boyce and Havill, 2005; Schmitz et al., 1997). Among food-borne diseases, which was estimated by the Centers for Disease Control (CDC) to affect approximately 76 million individuals resulting in 325,000 hospitalizations and 5000 deaths in the US alone (Mead et al., 1999), staphylococcal enterotoxin-contaminated food was reported to be the second most common cause (Pinchuk et al., 2010). SEB exposure, when ingested or inhaled, can produce mild food poisoning-like symptoms to more severe and potentially fatal conditions, such as toxic shock syndrome (Henghold, 2004).

SEB is an extremely potent antigen, classified as a superantigen, which bypasses normal processing by antigen-presenting cells (APCs), and results in nonspecific binding of the major histocompatibility complex class II (MHC-II) molecule on APCs with the variable region of the β chain of the T cell receptor (TCR) on T cells. This nonspecific binding leads to rapid T cell activation and uncontrolled release of cytokines, also referred to as a cytokine storm, producing an adverse inflammatory response (Baker and Acharya, 2004). It is estimated that while exposure of normal antigens can result in the activation of approximately 0.1% of host T cells, SEB exposure to the host can lead to the activation of 5 to 30% T cells (Reider et al., 2011). In addition to the robust activation of T cells, SEB was found to be remarkably stable in acidic environments, such as in the gastrointestinal tract, and highly resistant to both heat and proteolytic digestion (Ler et al., 2006). These properties of SEB, in addition its ability to become easily aerosolized, led the CDC to classify SEB as a category B priority agent for the potential use as a biological warfare weapon (Henghold, 2004). All of these factors illustrate the

Abbreviations: SEB, staphylococcal enterotoxin B; I3C, indole-3-carbinol; DIM, 3,3′-diindolylmethane; HDAC, histone deacetylase; HDAC-I, class I histone deacetylase; HDAC-II, class II histone deacetylase; TSA, trichostatin A; MG, MGCD0103; MC, MC1568.

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importance of discovering new therapies that would counteract the effects of SEB exposure. Inasmuch as, conventional antibiotic therapy may seem futile, given the emergence of the highly antibiotic-resistant strain of *S. aureus*, it would seem more appropriate to seek out treatments that could reduce the rapid T cell activation and inflammatory response caused by exposure of SEB to the host. In the current study, we investigated the potential role of naturally-occurring indole compounds, indole-3-carbinol (I3C) and one of its byproducts, 3,3′-diindolylmethane (DIM), in suppressing inflammation triggered by SEB.

I3C is an indole compound found in cruciferous vegetables, such as cabbage and broccoli, which is formed by the enzymatic breakdown of glucosinolate glucobrassicin by myrosinase. In acidic environments, I3C undergoes rapid self-condensation reactions that produce a variety of byproducts, with a major component being DIM (Aggarwal and Ichikawa, 2005). In terms of structure, DIM is formed by the combination of two I3C molecules (Fig. 1A) (Sarkar and Li, 2004). I3C and DIM have gained significant attention in the past based on their well-studied anti-cancer effects (Ahmad et al., 2010). However, the role of these compounds in exerting anti-inflammatory effects has emerged more recently (Busbee et al., 2013). DIM was shown to reduce the pro-inflammatory cytokines during dextran sodium sulfate (DSS)-induced experimental colitis in mice (Kim et al., 2009). Recent studies from our laboratory demonstrated that in experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis, both I3C and DIM ameliorated the clinical symptoms by reducing the infiltration of T cells into the brain, as well as decreasing the pro-inflammatory cytokines in the serum of diseased mice (Rouse et al., 2013).

Histone acetylation is an epigenetic modification that is regulated through histone deacetylases (HDACs). The role of HDACs involves the removal of acetyl groups on lysine residues, and in the case of histone proteins, this action plays a key role in the regulation of gene transcription (Haberland et al., 2009; Strahl and Allis, 2000). The role of HDACs in SEB-induced inflammation as well as the anti-inflammatory properties of dietary indoles has not been previously investigated. In the present study, we investigated the efficacy of I3C and DIM in reducing the activation of T cells stimulated with SEB, with particular emphasis on the role of HDACs. Our data demonstrate for the first time that HDACs play a prominent role in the promotion of activation and pro-inflammatory cytokine release following SEB stimulation. Also, I3C and DIM suppress SEB-induced inflammation by acting as HDAC inhibitors.

Materials and methods

Animals. Female C57BL/6 mice (aged 8–10 weeks) were purchased from the National Cancer Institute. All mice were housed at the AAALAC-accredited animal facility at the University of South Carolina, School of Medicine (Columbia, SC). All procedures were performed according to NIH guidelines under protocols approved by the Institutional Animal Care and Use Committee.

Effects of I3C and DIM on mice stimulated with SEB in vivo. To test the efficacy of treatment of I3C and DIM in an in vivo SEB mouse model, SEB, in sterile phosphate-buffered saline (PBS), was injected into each hind footpad of mice (10 μg/footpad) only once, as previously described

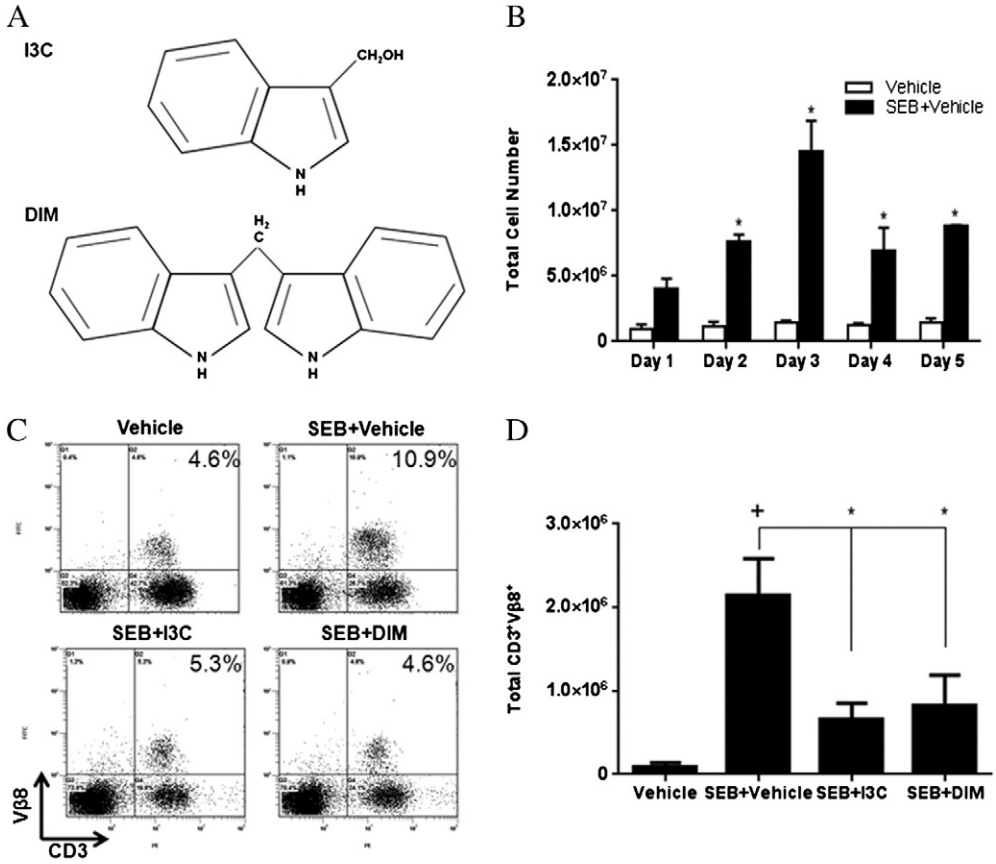

Fig. 1. Treatment with I3C or DIM in vivo reduces percentage and number of SEB-specific Vβ8 T cells. (A) Chemical structure of I3C and DIM. (B) C57BL/6 mice were given injections of 10 μg of SEB in each hind footpad only once. Total cellularity from popliteal lymph nodes isolated from vehicle-treated versus SEB-treated mice was depicted. Mice were given ip injections of I3C or DIM (40 mg/kg) for three consecutive days prior to SEB injection, which was followed by I3C and DIM treatment every other day. During the peak period of cell expansion on day 3, percentages (C) and total cell numbers (D) of $CD3^+$ $V\beta8^+$ from popiteal lymph nodes were determined in each experimental group ($n = 5$) using flow cytometry and antibodies for the respective markers. Statistical significance (p-value <0.05) was determined using GraphPad Prism analysis software with one-way ANOVA and Tukey's multiple comparison test (+ indicates significance compared to Vehicle group, and * indicates significance compared to SEB + Vehicle).