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Chronic psychological stress suppresses contact hypersensitivity: Potential roles of dysregulated cell trafficking and decreased IFN-γ production

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ABSTRACT

Increasing evidence shows that psychological stress can have dramatic impacts on the immune system, particularly the cutaneous immune response in dermatological disorders. While there have been many studies examining the impact of acute psychological stress on contact hypersensitivity there are relatively few studies concerning the impact of chronic psychological stress. Furthermore, the local immunological mechanisms by which chronic psychological stress impacts contact hypersensitivity still remain to be explored. Here we show that restraint-induced chronic psychological stress stimulates activation of the hypothalamus–pituitary–adrenal axis and delays weight gain in female BALB/c mice. We observed that chronic psychological stress reduces the cutaneous immune response as evidence by reduced ear swelling. This correlated with a significant decrease in the inflammatory cell infiltrate. On the other hand, chronic psychological stress does not influence T cell proliferation, activation, or sensitivity to corticosterone but does increase $CD4^+$ and $CD8^+$ T cell percentages in draining lymph nodes during a contact hypersensitivity reaction. Chronic psychological stress induces a decrease in overall circulating white blood cells, lymphocytes, and monocytes during a contact hypersensitivity reaction suggesting extravasation from the circulation. Finally, we found markedly reduced local IFN-γ production in chronically stressed animals. Based on these findings we propose that chronic psychological stress reduces contact hypersensitivity due to dysregulated cell trafficking and reduced production of IFN-γ.

1. Introduction

Psychological stress has been reported to impact various physiological functions in living animals, primarily by altering the nervous and endocrine systems. Furthermore, accumulating evidence supports the concept that generation of psychological stress can hinder or exacerbate the immune response, thus affecting disease outcome or disease manifestation (Priyadarshini and Aich, 2012). The skin represents the largest bodily organ and acts as the first line of defense against daily environmental insults. Additionally, there is interplay between the cutaneous immune response and psychological stress (Altemus et al., 2006; Dhabhar, 2009). In fact, 30% of psychiatric patients show a co-occurrence of skin disease and 10% of patients in one dermatology clinic were shown to have psychosomatic disorders (Shenefelt, 2011).

Allergic contact dermatitis (ACD) is a widespread skin disease that is responsible for 20% of work-related health illnesses and results in 4 million lost work days per year with an annual cost of $400 million in the United States (Kaplan et al., 2012; Thyssen et al., 2007). In fact, ACD accounts for one of the highest rates of work-related skin disease (Karsak et al., 2007; Martin, 2012), and work-associated exacerbation of skin sensitivity is increasing in developed countries (Nakano, 2007). Contact hypersensitivity (CHS) is a model of this allergic response. CHS is a delayed-type hypersensitivity response which results from exposure to low-molecular weight chemical haptens. By themselves, haptens cannot directly initiate an inflammatory response, but binding to host proteins activates damage-associated molecular pattern receptors and/or initiates breakdown of high-molecular weight hyaluronic acid to low molecular weight hyaluronic acid, activating pattern recognition receptors (Esser et al., 2012). Local dendritic cells take up the hapten-carrier complex and travel to draining lymph nodes

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to prime an antigen specific T-cell response (Kripke et al., 1990). This is termed the sensitization or afferent phase. In the effector or challenge phase, re-exposure to the chemical hapten elicits an inflammatory response mediated by T helper 1 (Th1) and $CD8^+$ T cells resulting in further cell recruitment and release of pro-inflammatory mediators (Oliver et al., 2011). Activated lymph nodes may primarily serve to conserve increased numbers of T cells in the effector phase (Fransen et al., 2010). Like many skin diseases, CHS has been shown to be particularly responsive to psychological stress and has been used to study the impact of psychological stress on the cutaneous immune response in rodent models (Dhabhar, 2000; Flint et al., 2001; Oliver et al., 2011).

Although stress is generally believed to suppress the immune response, conflicting reports have been generated, leading to the distinction between "acute" periods of stress and "chronic" periods of stress (Neeman et al., 2012). Acute stress has been defined as single events of stress that last for several minutes to hours (Dhabhar et al., 2012) and has been shown to enhance the cutaneous immune response in rodent models of CHS (Dhabhar, 2002, 2003; Flint et al., 2000). Enhancement correlates with a decrease in circulating T cells, B cells, natural killer (NK) cells, and monocytes by redistribution of these cells to the bone marrow, lymph nodes, and skin. Circulating neutrophils have been shown to be both elevated and diminished during CHS after periods of acute stress (Bowers et al., 2008; Dhabhar, 2003; Dhabhar et al., 2012). Additionally, several other skin diseases, including psoriasis (Griffiths and Richards, 2001; Janowski and Pietrzak, 2008) and atopic dermatitis (Hashizume and Takigawa, 2006; Kodama et al., 1999), have been shown to be aggravated by stressful events providing evidence for stress-mediated enhancement of the cutaneous immune response.

Chronic psychological stress has been defined as multiple events of stress that last several hours per day for a period of several weeks or months (Dhabhar et al., 2012). Fewer investigative efforts have focused on the effect of chronic psychological stress on CHS as compared with acute stress. Chronic psychological stress has been shown to suppress the immune response during CHS in rats as demonstrated by a decrease in the ear swelling response (Dhabhar and McEwen, 1997). However, the mechanism of CHS suppression induced by chronic psychological stress still remains to be fully explored.

Although a number of investigations have sought to assess hormonal factors that impact the ear swelling response, to our knowledge no one has sought to determine the locally occurring immunological mechanisms in the ear skin itself that are involved in suppressing the ear swelling response in chronic psychological stress. Our results show for the first time that chronically stressed mice exhibit reduced ear swelling responses potentially due to disrupted extravasation of inflammatory cells into the ear skin and reduced local production of cutaneous IFN-γ.

2. Materials and methods

2.1. Animals

Female BALB/c mice (4–6 weeks of age) were used for all experiments. Mice were housed 4 per cage at the University of North Texas Health Science Center. Animal experiments were performed in compliance with the U.S. Department of Health and Human Services Guide for the Care and Use of Laboratory Animals and all protocols were approved by the Institutional Animal Care and Use Committee (IACUC).

2.2. Chronic psychological stress model

A restraint stress model was used to induce chronic psychological stress similar to the method described by Bowers et al. (2008). Briefly, mice were confined in ventilated 50-mL conical tubes placed in the horizontal position for 2 h each day for a total of 30 days. During the stress period, both the stress and control groups were deprived access to food or water. Before and after the daily 2-h stress period, food and water was provided *ad libitum*. Weight was monitored daily. Restraint stress was performed at the same time each day and was not performed on the day of sacrifice.

2.3. Corticosterone

On day 0 and day 20 of the restraint stress period, tail blood was drawn from all mice to assess the concentration of circulating corticosterone similar to a previously described experiment (Bowers et al., 2008). Peripheral blood from the lateral tail vein was collected via heparin-coated glass capillary tubes. Blood was allowed to coagulate and serum was isolated by centrifugation. Corticosterone was evaluated by the Corticosterone EIA Kit (Enzo Life Sciences) according to the manufacturer's instructions.

2.4. Contact hypersensitivity

On day 25 of restraint stress, all mice were sensitized with oxazolone (Sigma–Aldrich). Briefly, an area of 2 × 2 cm was shaved on the abdomen of mice. Via pipette, 100 μL of oxazolone [1.25% (wt./vol) in 3:1 acetone:olive oil vehicle] was applied to the abdomen. 5 days after sensitization, the mice were challenged on the right pinna. Via pipette, 12.5 μL of oxazolone [0.3% (wt./vol) in 3:1 acetone:olive oil vehicle] was applied to both outer and inner surfaces of the ear. The left ear was treated with 12.5 μL of the 3:1 acetone:olive oil vehicle only on both outer and inner surfaces of the pinna. Ear thickness was measured with calipers 24 h after challenge (i.e., the peak of the ear swelling response) on day 31.

2.5. H&E histology and assessment

Vehicle and challenged ear from the control and stressed groups were harvested on day 31 of restraint stress. The tissue was fixed in paraformaldehyde, embedded in paraffin, sectioned 5 μm thick and processed with hematoxylin and eosin (H&E) (DermPrep Tampa, FL). The inflammatory cell infiltrate was assessed and quantified by visualization with an Olympus IX71 inverted research microscope.

2.6. In vitro T cell proliferation and glucocorticoid sensitivity

Spleens were harvested on day 31 of restraint stress. T cells were isolated utilizing the Mouse T-cell Enrichment Column Kit (R&D Systems) according to the manufacturer's instructions. Cells

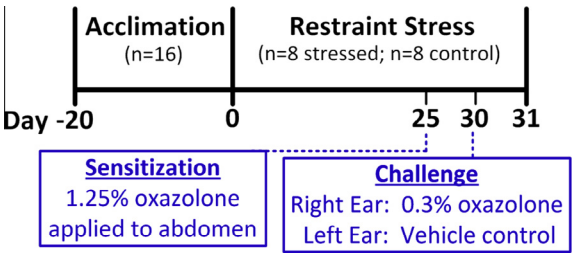

Fig. 1. Experimental model for restraint stress and induction of CHS. Female BALB/c mice received at 4 weeks of age were acclimated to the environment for 20 days. Chronic restraint stress was performed for 2 h each day for a total of 30 days. On day 25 of restraint stress, all mice were sensitized on the abdomen with oxazolone. On day 30 of restraint stress, all mice were challenged on the right ear with oxazolone and on the left ear with vehicle alone. Ear swelling was measured 24 h after challenge on day 31.