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Immune status influences fear and anxiety responses in mice after acute stress exposure

Sarah M. Clark [a,h], Joseph Sand [a], T. Chase Francis [b], Anitha Nagaraju [a], Kerry C. Michael [a], Achsah D. Keegan [c,h], Alexander Kusnecov [d], Todd D. Gould [b,e,f,g,h], Leonardo H. Tonelli [a,b,h,*]

[a] Laboratory of Behavioral Neuroimmunology, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, United States
[b] Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD, United States
[c] Center for Vascular and Inflammatory Diseases, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, United States
[d] Department of Psychology, Rutgers University, Newark, NJ, United States
[e] Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, United States
[f] Department of Pharmacology, University of Maryland School of Medicine, Baltimore, MD, United States
[g] Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD, United States
[h] Research and Development Service, Department of Veterans Affairs, VA Maryland Health Care System, Baltimore, MD, United States

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ABSTRACT

Significant evidence suggests that exposure to traumatic and/or acute stress in both mice and humans results in compromised immune function that in turn may affect associated brain processes. Additionally, recent studies in mouse models of immune deficiency have suggested that adaptive immunity may play a role during traumatic stress exposure and that impairments in lymphocyte function may contribute to increased susceptibility to various psychogenic stressors. However, rodent studies on the relationship between maladaptive stress responses and lymphocyte deficiency have been complicated by the fact that genetic manipulations in these models may also result in changes in CNS function due to the expression of targeted genes in tissues other than lymphocytes, including the brain. To address these issues we utilized mice with a deletion of recombination-activating gene 2 (*Rag2*), which has no confirmed expression in the CNS; thus, its loss should result in the absence of mature lymphocytes without altering CNS function directly. Stress responsiveness of immune deficient *Rag2*$^{-/-}$ mice on a BALB/c background was evaluated in three different paradigms: predator odor exposure (POE), fear conditioning (FC) and learned helplessness (LH). These models are often used to study different aspects of stress responsiveness after the exposure to an acute stressor. In addition, immunoblot analysis was used to assess hippocampal BDNF expression under both stressed and non-stressed conditions. Subsequent to POE, *Rag2*$^{-/-}$ mice exhibited a reduced acoustic startle response compared to BALB/c mice; no significant differences in behavior were observed in either FC or LH. Furthermore, analysis of hippocampal BDNF indicated that *Rag2*$^{-/-}$ mice have elevated levels of the mature form of BDNF compared to BALB/c mice. Results from our studies suggest that the absence of mature lymphocytes is associated with increased resilience to stress exposure in the POE and does not affect behavioral responses in the FC and LH paradigms. These findings indicate that lymphocytes play a specific role in stress responsiveness dependent upon the type, nature and intensity of the stressor.

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1. Introduction

Pathological responses to stress, as a result of a traumatic event, are known to be related to a combination of genetic and environmental factors that determine susceptibility or resilience to develop exacerbated fear responses (Gillespie et al., 2009; Skelton et al., 2012). Recent research suggests that impairments in immune function may be a central mechanism determining susceptibility or resilience to the development of these responses

* Corresponding author. Address: Laboratory of Behavioral Neuroimmunology, Department of Psychiatry, University of Maryland School of Medicine, Research & Development Service, VA Maryland Health Care System, 685 West Baltimore Street, MSTF Building Room 934 C, Baltimore, MD 21201, United States. Tel.: +1 410 706 2325.
E-mail addresses: ltonelli@psych.umaryland.edu, tonelli.leonard@va.gov (L.H. Tonelli).

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(Baker et al., 2012; Neylan et al., 2011) with a higher incidence of maladaptive responses among those with pre-existing inflammatory conditions (LeardMann et al., 2009; O'Toole and Catts, 2008). Additionally, a number of studies have shown a myriad of immune abnormalities, along with specific epigenetic modifications in genes associated with immune responses, in people suffering from conditions such as posttraumatic stress disorder (Glatt et al., 2013; Rusiecki et al., 2013; Smith et al., 2011; Uddin et al., 2010; Zovkic et al., 2013). This link between stress exposure and the immune system appears to be bi-directional, in which traumatic stress exposure is associated with a higher risk for developing a significant number of chronic inflammatory conditions (Lemieux et al., 2008; O'Toole and Catts, 2008; Plantinga et al., 2013). Despite mounting evidence implicating the immune system in pathological stress responses, specific mechanisms linking traumatic stress and immune function remain poorly understood.

Research using various animal models of immune deficiency suggests a role for the adaptive arm of the immune system in determining resilience to stress via mechanisms mediated through the actions of T cells (Cohen et al., 2006; Lewitus et al., 2008; Lewitus and Schwartz, 2009). These studies have found that the absence of adaptive immunity is associated with increased fear and anxiety responses after stress exposure (Cohen et al., 2006) and have culminated in the proposal that mature T cells help maintain homeostasis and confer protection against stress exposure by a mechanism involving down-regulation of pro-inflammatory cytokines and the production of brain-derived neurotrophic factor (BDNF) in the hippocampus (Lewitus et al., 2008; Lewitus and Schwartz, 2009; Schwartz and Ziv, 2008). Nevertheless, certain behavioral traits seen in the immune deficiency models employed in these studies may be due to the expression of targeted genes in the CNS rather than in peripheral immune cells alone (Fang et al., 2013; Rattazzi et al., 2013). Of particular concern is recombination-activating gene 1 (RAG1), which is highly expressed in the hippocampus and cerebellum, as well as lymphocytes (Chun et al., 1991) and whose deletion induces behavioral deficits independent of lymphocyte function (Fang et al., 2013; McGowan et al., 2011).

The purpose of the present study was to further clarify the role of the adaptive immune system in stress responsiveness by employing the *Rag2*$^{-/-}$ mouse model of immune deficiency. Similar to *Rag1*$^{-/-}$ mice, the loss of RAG2 in these mice inactivates the variable (diverse) recombination (V[D]J) process of the immunoglobulin and T cell and B cell receptors. However, in contrast to RAG1, RAG2 has no confirmed expression in the CNS (Chun et al., 1991; Shinkai et al., 1992) (Supplemental Figs. 1 and 2) and thus, the impact of its loss should be restricted to peripheral lymphocytes. As a result, *Rag2*$^{-/-}$ mice lack mature T and B lymphocytes while maintaining normal hematopoiesis (Shinkai et al., 1992). To examine the role of lymphocytes in several models of stress responsiveness after acute stress exposure, mice were tested in the following fear and stressor paradigms: (1) predator odor exposure (POE), (2) Pavlovian fear conditioning (FC), and (3) learned helplessness (LH). Additionally, the expression of BDNF was examined in order to explore the potential relationship between lymphocytes, stress exposure and the regulation of this neurotrophic factor. Our results indicate that immune deficient and immune competent mice display similar behavior in the FC and LH paradigms while the absence of lymphocyte function contributes to resilience in the POE paradigm. Moreover, hippocampal BDNF levels for the mature form of the protein were higher in *Rag2*$^{-/-}$ mice under basal conditions and following LH, but not POE. The present studies suggest that the impact of lymphocyte function on stress responsiveness is dependent on the nature of the stressor and type of response involved.

2. Methods

2.1. Animals

Rag2$^{-/-}$ mice were originally developed by the Alt laboratory by targeting the RAG2 gene in CCE embryonic stem cells and transferring targeted cells into blastocysts (Shinkai et al., 1992). For this study, *Rag2*$^{-/-}$ mice were acquired from Taconic Farms, Inc. (Hudson, NY) where they have been backcrossed onto the BALB/c background for twelve generations and maintained by homozygous pairings. Male *Rag2*$^{-/-}$ and wild type (WT) BALB/c mice were obtained at 5–6 weeks of age and housed under normal conditions (12 h light/dark cycle, 4–5 mice per cage) with *ad libitum* access to food and water. All experiments were conducted when mice were between 8–12 weeks old. Prior to beginning any experiments all animals were handled daily for several days to habituate the animals to the experimenter and to monitor overall health. Any cages exhibiting severe signs of fighting between cage mates either before or after stress exposure were excluded. Verification of immune status was conducted by flow cytometry for all mice at the completion of each experiment (Supplemental Fig. 3). All procedures were carried out under approved IACUC protocols and institutional guidelines at the University of Maryland, School of Medicine and Baltimore VA Health Care System.

2.2. Basal behavioral assessments

To determine if basal behavioral responses were comparable between WT and *Rag2*$^{-/-}$ mice, an independent group of animals was first tested in the open field test (day 1) followed by the elevated plus maze test (day 2). In addition, to ensure that the olfactory system was not compromised by the absence of lymphocytes a group of mice was evaluated in a buried food test. All tests were conducted between 10 am and 3 pm under 5 lux illumination and constant background white noise (~65–70 dB).

2.2.1. Open field test (OFT)

Individual mice (WT: $n = 12$; *Rag2*$^{-/-}$: $n = 14$, 8 weeks old) were placed in square arenas (50 × 50 cm) and allowed to explore for 30 min while being recorded overhead. Total distance traveled and time in center (interior 50% of the arena) were analyzed with the use of TopScan (Cleversys; Reston, VA).

2.2.2. Elevated plus maze test (EPM)

The EPM is an apparatus raised 50 cm above the ground with two enclosed arms (35 × 5 × 15 cm) perpendicular to two open arms (39.5 × 5 cm) intersected by an open central area (5 × 5 cm). Individual mice were placed in the center facing one of the two open arms and recorded with an overhead camera for 10 min as they freely explored the maze. An observer was present for the entire session and any mouse that fell off of the maze was returned to the same arm, in the same position. TopScan was used to determine total distance traveled, the number of entries into each arm and the proportion of time spent in the open arms.

2.2.3. Olfaction test

To evaluate whether there are differences in olfaction between WT and *Rag2*$^{-/-}$ mice that may influence the effect of predator odor exposure, a buried food test (Yang and Crawley, 2009) was conducted to compare the latency of WT ($n = 8$) and *Rag2*$^{-/-}$ mice ($n = 9$) to sniff out and begin consuming a treat that they had been familiarized with in their home cage. For the test a Honey Teddy Graham (Nabisco) was buried in the bedding at one end of a clean cage and a single mouse was placed at the opposite end of the cage. The animal was then allowed to freely explore the cage for 15 min;

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