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Therapeutic effects of stress-programmed lymphocytes transferred to chronically stressed mice

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ABSTRACT

Our group has recently provided novel insights into a poorly understood component of intercommunication between the brain and the immune system by showing that psychological stress can modify lymphocytes in a manner that may boost resilience to psychological stress. To demonstrate the influence of the adaptive immune system on mood states, we previously showed that cells from lymph nodes of socially defeated mice, but not from unstressed mice, conferred anxiolytic and antidepressant-like effects and elevated hippocampal cell proliferation when transferred into naïve lymphopenic Rag2$^{-/-}$ mice. In the present study, we asked whether similar transfer could be anxiolytic and antidepressant when done in animals that had been rendered anxious and depressed by chronic psychological stress. First, we demonstrated that lymphopenic Rag2$^{-/-}$ mice and their wild-type C57BL/6 mouse counterparts had similar levels of affect normally. Second, we found that following chronic (14 days) restraint stress, both groups displayed an anxious and depressive-like phenotype and decreased hippocampal cell proliferation. Third, we showed that behavior in the open field test and light/dark box was normalized in the restraint-stressed Rag2$^{-/-}$ mice following adoptive transfer of lymph node cells from green fluorescent protein (GFP) expressing donor mice previously exposed to chronic (14 days) of social defeat stress. Cells transferred from unstressed donor mice had no effect on behavior. Immunolabeling of GFP+ cells confirmed that tissue engraftment had occurred at 14 days after transfer. We found GFP+ lymphocytes in the spleen, lymph nodes, blood, choroid plexus, and meninges of the recipient Rag2$^{-/-}$ mice. The findings suggest that the adaptive immune system may play a key role in promoting recovery from chronic stress. The data support using lymphocytes as a novel therapeutic target for anxiety states.

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1. Introduction

Mental health is affected by bidirectional communication between the central nervous system and the immune system (Maier and Watkins, 1998). The two systems normally coexist in homeostasis. Loss of homeostasis results in a disequilibrium that can precipitate mental and physical disease (Gibney and Drexhage, 2013; Slavich and Irwin, 2014). Understanding the details of this communication system might lead to development of therapies directed at non-traditional targets—perhaps within particular compartments of the immune system—that promote restoration of homeostasis.

We have been exploring the link between mood changes and immune system function in a rodent model of chronic psychosocial stress. Whereas the usual focus of neuroimmune research of this type is the innate immune system and its associated production of inflammatory cytokines (Slavich and Irwin, 2014), the role of the adaptive immune system, represented mainly by the activity of T lymphocytes, has begun to attract recent attention (Miller, 2010). We (Brachman et al., 2015) and others (Lewitus et al., 2009) have hypothesized that the adaptive immune system, represented by lymphocytes residing in lymph nodes throughout the body, is altered by stressful events in the animal and retains a "memory" for those events. In our recent study, the contribution of stress-conditioned lymphocytes to mood states was tested by transferring those cells into a naïve mouse that lacked an adaptive immune system—the lymphopenic Rag2$^{-/-}$ mouse. We found that lymphocytes from chronically stressed mice adoptively transferred into naïve Rag2$^{-/-}$ mice conferred anxiolytic and antidepressant-like behavioral effects and elevated hippocampal cell proliferation rates whereas lymphocytes transferred from unstressed home-caged mice had no effect on behavior or cell proliferation (Brachman et al., 2015).

These surprising findings suggested that lymphocytes, known to carry an immunological memory for prior immunological insults that allows them to counter the effects of the insults, might also have a memory for prior adverse psychological events and thus contribute in a homeostatic fashion to counter the deleterious effects of stress on

Abbreviations: BrdU, ((+)-5-bromo-2-deoxyuridine); GFP, green fluorescent protein; HC, Home-cage; L/D, light/dark; OFT, open field test; Rag2, recombination activating gene 2; SD, social defeat; TST, tail suspension test; UBC, human ubiquitin C.

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the brain and behavior. However, the animals that received the stress-programmed lymphocytes were naïve, so it was difficult to conclude that the apparently beneficial effects could be accurately called "antidepressant" and "anxiolytic." To directly address this concern, we sought to determine whether beneficial effects might occur in mice that had been rendered "anxious and depressed" by chronic psychological stress. In this study, therefore, we characterized the ability of Rag2$^{-/-}$ mice to become affectively impaired by repeated restraint stress and whether such impairments could be reversed by lymphocytes adoptively transferred from stressed and/or unstressed donor mice. We concomitantly measured new cell proliferation in the hippocampal dentate gyrus as an index of activity in a key component of a neural circuit associated with control of affective behavior (Egeland et al., 2015; Kheirbek et al., 2012; Samuels and Hen, 2011). Finally, as a preliminary investigation of mechanism of effect, we tracked and partially characterized the distribution of transferred lymph node cells in the recipient mice by using donor mice that expressed green fluorescent protein (GFP) in all cells.

2. Materials and methods

2.1. Subjects

Adult (8–12 weeks) male wild-type C57BL/6 mice (n = 16) and Rag2$^{-/-}$ mice (008449, strain B6(Cg)-Rag2$^{tm1.1Cgn}$/J; n = 32) were used in the first experiment, and donor UBC-GFP mice (004353, strain C57BL/6-Tg(UBC-GFP)30Scha/J; n = 18) and recipient Rag2$^{-/-}$ mice (n = 26) were used in the second experiment. All mice were originally from The Jackson Laboratory with a C57BL/6 background and bred in house. The Rag2$^{-/-}$ mice, which lack mature B and T cells, were housed in sterilized cages with sterilized food and water. At least one week prior to the start of and throughout the experiment, all mice were housed in a reverse 12 h light/dark cycle (lights on from 9:00 pm to 9:00 am), and procedures were performed during the dark cycle under dim illumination. All animal procedures were conducted in accordance with NIH guidelines and were approved by the NIMH Institutional Animal Care and Use Committee.

2.2. Procedures

2.2.1. Restraint

We did not subject Rag2$^{-/-}$ mice to chronic social defeat (SD) stress, a paradigm that we have used previously to model a depressive-like state, because the interaction with another animal would pose an immunological challenge and health risk. Instead, chronic restraint stress was used to produce a similar behavioral outcome. At 11:00 am (±2 h), singly housed Rag2$^{-/-}$ (n = 16) and C57BL/6 (C57) mice (n = 8) were placed in perforated 50 mL conical Falcon tubes inside their home cages for 4 h each day for 14 days. Mice were monitored throughout the experiment for changes in weight and overall health. Control home-caged (HC) mice were singly housed for the same period and not restrained, but their water bottles were removed for the 4-h duration.

2.2.2. Social defeat (SD)

Donor UBC-GFP mice were exposed to chronic SD stress to induce a depressive-like behavioral phenotype as previously described (Brachman et al., 2015; Lehmann and Herkenham, 2011). Briefly, mice were housed for 14 days in dyads comprising the experimental intruder mouse with a resident, larger, older, and more aggressive male CD-1 mouse (The Jackson Laboratory). A perforated Plexiglas divider separating the pair was removed for 5 min each day. Bouts were monitored and scored based on conflicts won and lost, exploration, hiding, and freezing. Mice were swapped between resident cages when the resident CD-1 mouse was not showing aggressive behavior or the UBC-GFP mouse was not demonstrating defeat behavior to ensure that all experimental mice received 5 min of agonist interactions during each SD exposure period. HC control mice were group-housed for the same period of time in standard cages with bedding, nestlets, and cardboard tubes.

2.2.3. Adoptive transfer

SD or HC UBC-GFP donor mice were euthanized by CO_2 asphyxiation. Cells were harvested from the cervical, axillary, inguinal, and mesenteric lymph nodes and passed through a 70-μm filter to produce a single-cell suspension in sterile cell sorting medium (145 mM NaCl, 5 mM KCl, 1.8 mM CaCl2, 0.8 mM MgCl2, 10 mM Hepes, 10 mM glucose and 1 mg/L BSA; pH 7.3 and 290–200 mOsm). Cells were pooled by housing condition (HC or SD) and resuspended in sterile saline. The restraint-stressed Rag2$^{-/-}$ mice were injected (0.15 cc, i.p., a route recommended by R. Caspi, personal communication and (Tasso et al., 2012)) with 20 million HC cells (n = 6), SD cells (n = 12), or sterile saline (n = 8) under mild isoflurane anesthesia.

2.2.4. Behavioral analysis

Beginning 11 d after adoptive transfer, restraint-stressed recipient Rag2$^{-/-}$ mice were behaviorally characterized on three tests, one test per day, using automated digital tracking systems (TopScan and TailSuspScan; Cleversys) as previously described (Lehmann et al., 2013).

2.2.4.1. Open-field test (OFT). Mice were placed in an empty Plexiglas box (50 × 50 × 50 cm) for 30 min and recorded from above. Time spent in the middle 50% of the arena was recorded as a measure of anxiolytic-like behavior.

2.2.4.2. Light/dark test (L/D). Mice were placed in the light compartment of a Plexiglas box (50 × 25 × 30 cm) that was subdivided into a one-third dark (safe) area and a two-thirds light (aversive) area. Activity was recorded from above for 10 min. Time spent in the light and the number of crosses between the two open compartments was used as anxiolytic measures.

2.2.4.3. Tail suspension test (TST). Mice were suspended by their tails with adhesive tape 60 cm above a surface. Mice were recorded for 6 min and the last 5 min were scored for mobility.

2.2.5. BrdU administration, perfusion and tissue harvest

The day following TST, mice were injected with the cell-synthesis marker BrdU ((+)-5-bromo-2-deoxyuridine; Sigma Aldrich, 200 mg/kg, i.p.). Three hours after BrdU administration, mice were deeply anesthetized with isoflurane and chloropent (mixture of pentobarbital and chloral hydrate, 0.15 mL, i.p.) and perfused transcardially with 16 mL 0.9% saline and 12 mL cold 4% paraformaldehyde in phosphate buffer (PB). Spleens and brains were harvested, stored overnight in 4% paraformaldehyde/PB and equilibrated in 50% sucrose for 24 h before sectioning for immunohistochemical analysis. Brains were sliced coronally on a sliding microtome at 50 μm through the rostral–caudal extent of the hippocampus and collected serially.

2.2.6. BrdU immunohistochemistry

Every sixth section of the brain was mounted and dried onto slides prior to immunostaining. Sections were incubated for 15 min in 10 mM sodium citrate buffer at 95°C, treated with 0.9% H_2O_2 to quench endogenous peroxidases, blocked in 6% normal goat serum and incubated overnight at 4 °C in rat anti-BrdU (Sigma, OTB0030; 1:500). The following day, sections were incubated for 1 h in biotinylated anti-rat IgG (1:500) and treated with VectaStain avidin-biotin horseradish peroxidase kit (PK6100) for 1 h and Ni-DAB substrate kit according to instructions (both from Vector Laboratories) to reveal BrdU-labeled cells. Sections were washed in phosphate buffered saline (PBS) between