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

Rachel B. Scheinert, Mitra H. Haeri, Michael L. Lehmann, and Miles Herkenham*

Section on Functional Neuroanatomy, Intramural Research Program, National Institute of Mental Health, NIH, Bethesda, MD, USA

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.

Keywords

Lymphocytes; Psychological stress; Restraint; Depression; Hippocampal neurogenesis

*Corresponding author at: Bldg. 35, Rm. 1C913, Bethesda, MD 20892-3724. herkenh@mail.nih.gov (M. Herkenham).

Disclosures

All authors approved the final article. MHH and MLL contributed to the study design. RBS, MHH, and MLL collected and analyzed the data. MH and RBS supervised the work. MH and RBS wrote the manuscript. All authors declare no conflicts of interest.

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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, Lehmann, 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 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 hours), 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, Lehmann, 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 CO₂ asphyxiation. Cells were harvested from the cervical, axillary, inguinal, and mesenteric lymph nodes and passed through a 70- μ m 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 minutes. 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% H₂O₂ 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 one hour in biotinylated anti-rat IgG (1:500) and treated with VectaStain avidin-biotin horseradish peroxidase kit (PK6100) for one 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 all steps. Following alcohol dehydration, stained sections were cover-slipped with Permount.

2.2.7. Stereological estimate of hippocampal cell proliferation—Dividing (BrdU-labeled) cells were counted in the dentate gyrus subgranular layer of both the left and right hippocampi on every sixth section (8 sections per brain) on a Leica DMR microscope with a 40x objective. Dentate gyrus lengths (distance tip-to-tip on the inside of each blade) were measured using StereoInvestigator software on a Nikon Eclipse E800 microscope with a 10x objective. Dentate area was calculated by multiplying length by 50 μm —the average dentate thickness, and dentate volume was further estimated by using the equation $\frac{1}{3} I \times (h_1 + h_1 \times h_2 + h_2)$, where I is the interval between adjacent sections (300 μm) with dentate areas h_1 and h_2 . Hippocampal cell proliferation is expressed as an estimated density of BrdU cells per cubic mm of DG.

2.2.8. Fluorescent immunohistochemistry—Brain sections cut as above and spleen sections that were cryostat-cut at 20 μm -thick and slide-mounted were washed in PBS, blocked in 3% normal goat serum, incubated overnight at 4° C in rabbit anti-GFP (Santa Cruz, SC8334; 1:1000) and rat anti-CD3 (Serotec, MCA500GA; 1:500), washed in PBS, incubated for 4 h at room temperature in the secondary antibodies goat anti-rabbit conjugated with Alexa 488 (Invitrogen; A11034, 1:500) and goat anti-rat conjugated with Alexa 555 (Invitrogen; A21434, 1:500), and stained with DAPI (4',6-diamidino-2-phenylindole; Life Technologies; 1:10,000). Slides were cover-slipped with a polyvinyl alcohol mounting media with DABCO (1,4-Diazabicyclo[2.2.2]octane, Sigma) to prevent fading. Adoptive transfer and survival of GFP+ lymphocytes was confirmed with fluorescent confocal microscopy (Zeiss 780). Lymph nodes and blood collected from the atrium were spot-checked for presence of GFP+ cells by preparing a small sample on a slide for immediate viewing with a fluorescent microscope (Leica DMR).

2.3. Statistical Analyses

Data were presented as means $\pm$ SEM and were analyzed using PRISM software. In Experiment 1, the total time in center on the OFT, the number of crosses and time in light on the L/D test, time mobile on the TST, and hippocampal proliferation were analyzed using a two-way ANOVA with multiple comparisons followed by Tukey's post-hoc analysis when appropriate. In Experiment 2, the total time in center on the OFT, the number of crosses and time in light on the L/D test, time mobile on the TST, and hippocampal proliferation were analyzed using a one-way ANOVA followed by Tukey's post-hoc analysis.

3. Results

3.1. Experiment 1: Characterization of C57 mice and Rag2^{-/-} mice without adoptive transfer

The first experiment characterized the effects of chronic restraint stress on Rag2^{-/-} and wild-type C57BL/6 mice. The timeline for Experiment 1 is shown in Figure 1a.

3.1.1 Like wild-type C57BL/6 mice, Rag2^{-/-} mice show increased anxiety following chronic restraint stress—Following 14 days of restraint stress and a 10-day recovery period, wild-type C57BL/6 mice and Rag2^{-/-} mice were behaviorally characterized on the OFT, L/D test, and TST. On the OFT, there was significant effect of treatment ($F_{(1,44)}$

= 17.5, $p < 0.0001$; Fig. 1b) with both C57BL/6 and Rag2^{-/-} restraint-stressed groups spending less time in the center of the arena. Similarly, restraint-stressed C57BL/6 and Rag2^{-/-} groups demonstrated anxiety-like behavior on the L/D test with a significant treatment effect indicating they made fewer number of crosses ($F_{(1,44)} = 38.7$, $p < 0.0001$; Fig. 1c) and spent less time in the light compartment ($F_{(1,44)} = 49.8$, $p < 0.0001$; Fig. 1d). Two-way ANOVA detected a significant interaction effect ($F_{(1,44)} = 8.04$, $p < 0.01$) apparently because the decline in time in light in the C57 group was larger than in the Rag2^{-/-} group. Post-hoc tests showed significant stress ($p < 0.01$) but not strain effects ($p > 0.05$). In the TST, a test of behavioral despair, there was an overall main effect of treatment with stressed mice spending less time mobile ($F_{(1,43)} = 7.83$, $p < 0.01$; Fig. 1e).

Hippocampal cell proliferation, assessed by stereologically estimating the number of BrdU-labeled cells per cubic mm after a 3-h labeling procedure, was reduced in mice exposed to restraint stress ($F_{(1,44)} = 7.44$, $p < 0.01$; Fig. 2).

The weights of mice in the groups were not different, ranging between 24 and 28 g at the start of the experiment and between 25 and 30 g at the end of the experiment. Mice undergoing restraint stress lost about 2 g over the 14-day course, but recovered lost weight by the end of the experiment (data not shown). At autopsy, the spleens of all mice appeared normal in size.

3.2. Experiment 2: Characterization of stressed Rag2^{-/-} mice after adoptive transfer

Once we had demonstrated that Rag2^{-/-} mice showed normal affective behavior in the unstressed condition and a depressive-like behavioral phenotype following chronic restraint stress, we sought to reverse the stress effect by adoptive transfer of lymphocytes from HC or SD donor mice. The timeline for Experiment 2 is shown in Figure 3a. All Rag2^{-/-} mice in Experiment 2 displayed similar weight loss over the course of chronic restraint stress and subsequent weight gain when stress was stopped and following saline or cell transfer (data not shown).

3.2.1. Stressed mice that received adoptive transfer of lymphocytes from SD mice showed anxiolytic behaviors when compared to saline-treated and HC cell-recipient mice—Beginning ten days after adoptive transfer, stressed Rag2^{-/-} mice were behaviorally characterized on the OFT, L/D test, and TST. Mice that received lymphocytes from SD donors spent more time in the center of the arena in the OFT ($F_{(2,22)} = 9.253$, $p < 0.01$; Fig. 3b) compared to both saline-treated mice ($p < 0.01$) and mice that received lymphocytes from HC donors ($p < 0.01$). Treatment affected both the number of crosses ($F_{(2,23)} = 8.731$, $p < 0.01$; Fig. 3c) and time spent in the light ($F_{(2,23)} = 12.48$, $p < 0.001$; Fig. 3d) on the L/D test. SD AT mice spent significantly more time in the center of the OFT than both the Sal ($p < 0.01$) and HC AT ($p < 0.01$) mice. Interestingly, mice that received cells from HC mice had significantly fewer crosses and spent less time in time in light in the L/D test than both saline-treated mice and mice that received cells from SD donors ($p < 0.01$). There were no significant differences between treatment groups on the TST (Fig. 3e). Furthermore, treatment did not affect hippocampal cell proliferation (Fig. 3f).

3.3. Donor UBC-GFP cells populate the host at 14 days post-adoptive transfer

Using donor UBC-GFP mice that express GFP in all cells, we were able to track GFP+ donor lymphocytes to the secondary lymphoid tissues, blood, and brains of the recipient Rag2^{-/-} mice to confirm that transfer was successful and that cells survived 14 days post-adoptive transfer. No notable differences were seen between HC and SD donors at this cursory level of analysis. In the spleen (Fig. 4a), GFP+ cells were distributed mainly as clusters in the white pulp. In the brain, scattered cells were located in the leptomeninges (Fig. 4b) and choroid plexus, and only very rarely in the brain parenchyma (data not shown). Almost all GFP+ cells in and around the brain were co-labeled with CD3, the pan T lymphocyte marker (Fig. 4b). GFP+ cells were also identified in the lymph nodes and blood of recipient Rag2^{-/-} mice (data not shown).

4. Discussion

4.1. Experiment 1

The data in Experiment 1 showed that Rag2^{-/-} mice show basal levels of affective behavior that are not different from strain-matched C57BL/6 mice, and they succumb to chronic restraint stress by showing elevated anxiety at levels similar to control mice. Interestingly, the anxiety measured in the OFT and L/D test endured for 11–14 days after cessation of the restraint stress. The delay may have contributed to the failure to obtain a significant effect in the TST by post-hoc analysis. Alternatively, moderate levels of stress afforded by restraint in Falcon tubes placed in the home cages may not have been sufficient to induce depressive-like behavior and reduced hippocampal cell proliferation, both of which showed non-significant trends in control C57BL/6 and in Rag2^{-/-} mice. Thus, in all measures, the Rag2^{-/-} and wild-type mice were similar.

Experiment 1 data suggest that both normal affective function and response to adverse psychological events do not require an intact adaptive immune system. These findings challenge the contention that lymphocyte presence is needed for proper CNS function, including hippocampal neurogenesis, cognition, and mood (Baruch and Schwartz, 2013, Kipnis et al., 2004, Rook et al., 2011). Indeed, some studies have shown that T cell depletion in naïve wildtype mice can lead to reduced cognitive function and possibly changes in mood (Derecki et al., 2010, Rattazzi et al., 2013, Wolf et al., 2009). There have been some behavioral deficits noted in Rag2^{-/-} mice, i.e., acquisition and reversal in a Morris water maze (Radjavi et al., 2014) and deficit in acoustic startle in Rag2^{-/-} mice on BALB/c background (Clark et al., 2014), but there are no reports of deficits in affective tasks.

One difference between study outcomes is the nature of the task, but another may be the selection of type of lymphopenic mice. Although both Rag1 and Rag2 proteins are required for lymphocyte maturation (McBlane et al., 1995), and therefore knockout of either gene is sufficient for lymphocyte depletion, we did not use the Rag1^{-/-} mouse for study because the Rag1 gene but not the Rag2 gene is expressed in the brain, strongly in the hippocampus (Chun et al., 1991), which may lead to altered cognitive behavior in this mouse (Castro-Pérez et al., 2016, Cushman et al., 2003, McGowan et al., 2011, Rattazzi, Piras, 2013). The Rag1^{-/-} mouse also shows reduced adult hippocampal neurogenesis compared to wildtype

(Huang et al., 2010, Wolf, Steiner, 2009). Lymphopenic SCID mice show even more profound deficits in cognitive behavior and hippocampal neurogenesis basally and do not respond to treatments like environmental enrichment (Cohen et al., 2006, Kipnis, Cohen, 2004, Ziv et al., 2006). It has been reported that the SCID mutation causes a generalized defect in DNA repair that extends the cellular deficiencies beyond the loss of lymphocytes (Fulop and Phillips, 1990). Such additional actions may underlie the behavioral and CNS deficits. It was noted that nude mice, which are athymic and lack T cells, are as impaired in anxiety tests following exposure to predator odor as are SCID mice (Cohen, Ziv, 2006). They also have impaired neurogenesis basally (Ziv, Ron, 2006). Again, however, nude mice have additional organ deficiencies that might contribute to the behavioral defects. Our data suggest that lymphopenia from birth, as manifested in the Rag2^{-/-} mouse, *per se* does not contribute to changes in hippocampal neurogenesis and affective behavior.

4.2. Experiment 2

The data in Experiment 2 showed that the anxious phenotype in Rag2^{-/-} mice could be reversed by adoptive transfer of lymph node cells from SD but not HC mice. Transfer of cells from HC mice had no effect in the OFT and was actually anxiogenic in the L/D test, reproducing a similar finding in our original study (Brachman, Lehmann, 2015). There may be differences in level of anxiety in the two tasks that allow for effects to be revealed in one but not the other test. The new findings in stressed/anxious mice suggest that the adaptive immune system may play a key role in promoting recovery from chronic stress-induced anxiety. Unlike the original study in naïve Rag2^{-/-} mice, an anti-depressant-like effect of transfer was not found, possibly owing to the high level of mobility recorded in the saline control mice in the TST experiment. Alternatively, the depressive effect of restraint may have waned after 14 days following termination of the restraint sessions; the time-mobile scores in the stressed mice were similar to the scores of control mice in the first experiment. Finally, in this study, no effects on cell proliferation were found, whereas the earlier work showed that lymphocytes transferred from stressed but not unstressed mice elevated proliferation in naïve Rag2^{-/-} mice. Elevated neurogenesis is one possible mechanism by which lymphocytes might exert an anxiogenic effect, and the present data do not support this notion, though they do not rule it out either because the measure of cell proliferation taken at the late time point may not reflect earlier proliferation rates or changes in cell survival over the same period. Future experiments may examine cell proliferation difference in these two strains that have not undergone behavioral testing immediately following restraint stress.

It is noteworthy that the form of stress that induced the anxiety in the Rag2^{-/-} mice—restraint stress is—different than the stressor used to program the donors' immune cells—social defeat. The cross-paradigm anxiolytic efficacy of the stressed cells suggests that there are common mechanisms at play whereby the immune system responds to stress and achieves homeostasis in the brain circuits that process it.

4.3. Fate of transferred cells

GFP+ cells were found in spleen, blood, and barrier regions of the brain at 14 days after transfer. We found only occasional cells residing in the brain. They were mainly located in the choroid plexus and meninges, and very rarely in the brain parenchyma. The low number

may in part reflect the timing of the transfer. In lymphopenic hosts, lymphocytes undergo homeostatic proliferation within secondary lymphoid organs. Expansion occurs over the first weeks to months (Bell et al., 1987), and the final levels approximate but do not reach those of normal animals (Rocha et al., 1989). Scarce presence of non-CNS-reactive transferred lymphocytes in the brain parenchyma is a typical finding (Wolf, Steiner, 2009) and suggests that the mechanism whereby transferred cells alter CNS-mediated behavior may involve cellular and humoral mediators that interact with or cross the blood-brain and blood-cerebrospinal fluid barriers. Details of the cellular localization and the mechanisms of influence on brain will be the subject of future research.

5. Conclusion

Research investigating the relationship of adaptive immunity to psychological stress and stress-induced affective disorders is in its infancy. A major strategy thus far to explore that relationship is to characterize lymphopenic mice with and without adoptive transfer of lymphocytes or wildtype mice with depleted lymphocyte subsets. Despite the limited number of studies and different experimental approaches, mouse models, and behavioral paradigms employed, a clearer picture is starting to emerge supporting a beneficial role for lymphocytes in cognition and memory (Kipnis et al., 2012, Schwartz and Kipnis, 2011, Yirmiya and Goshen, 2011) and stress responsiveness and depressive states (Schwartz and Shechter, 2010, Segerstrom and Miller, 2004, Zorrilla et al., 2001). The research supports using lymphocytes as a novel therapeutic target as an anxiolytic strategy in treating depression and it suggests further that “programmed” lymphocytes could be used proactively as an “immunization” against stress effects. The idea was first proposed by Lewitus and Schwartz (2009) who suggested that exposure to mild stressors can program the adaptive immune system to afford resistance to later stressors. Future work will test the hypothesis that adoptive transfer of stressed lymphocytes made prior to the stressful events will protect host Rag2^{-/-} mice from their deleterious effects.

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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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Highlights

- Rag2^{-/-} mice lacking an adaptive immune system have normal affect and hippocampal cell proliferation
- Chronic restraint stress creates an anxious and depressed state in both Rag2^{-/-} and control mice
- Transfer of lymphocytes from stressed mice into stressed Rag2^{-/-} mice restores normal affect
- Transferred lymphocytes populate lymphoid organs, choroid plexus, and meninges

Figure 1.

a) Timeline: C57BL/6 and Rag2^{-/-} mice underwent chronic restraint stress or were housed in control conditions for 14 days. Starting on day 25, mice were behaviorally characterized in three tests done on consecutive days. The cell-synthesis marker BrdU was injected (200 mg/kg, i.p.) 3 h prior to sacrifice. b) Both C57BL/6 and Rag2^{-/-} restraint-stressed mice spent less time in the center of the arena than control mice on the open field test (OFT). c, d) On the light/dark test (L/D), restraint-stressed mice had fewer crosses and spent less time in light box. e) Restraint-stressed mice spent less time mobile than control mice on the tail suspension test (TST), significant as a main effect. Graphs show group average $\pm$ S.E.M. Post-hoc significance marked. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Figure 2.

a) Hippocampal cell proliferation was reduced in mice exposed to chronic restraint stress, significant as a main effect. **** $p < 0.01$** . b–e) Representative photomicrographs of BrdU-labeled cells in C57 and Rag2^{-/-} mice exposed to control or chronic restraint stress conditions. Magnification bar = 100 µm and 20 µm in the inset

Figure 3.

a) Experiment 2 timeline: donor UBC-GFP mice were exposed to home cage (HC) or social defeat (SD) at the same time that recipient Rag2^{-/-} mice underwent chronic restraint stress for 14 days. Lymphocytes harvested from HC or SD UBC-GFP mice were adoptively transferred into the Rag2^{-/-} mice, and a control group received saline. Starting on day 25, mice were behaviorally characterized for three days, and BrdU was injected 3 h prior to sacrifice. Stressed Rag2^{-/-} mice that received adoptive transfer from SD donor mice showed anxiolytic effects on the OFT (b) and L/D test (c and d), but no differences were revealed on the TST (e). Adoptive transfer did not affect hippocampal cell proliferation in stressed Rag2^{-/-} mice (f). Graphs show group average $\pm$ S.E.M. Post-hoc significance marked. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 4.

Donor UBC-GFP cells were tracked to the recipient Rag2^{-/-} spleens and brains to confirm adoptive transfer and cell survival 14 days later. a) Representative photomicrograph of GFP+ cells repopulating the spleen of a Rag2^{-/-} mouse 14 days after adoptive transfer of lymphocytes from **home cage (HC) donor**. b) Scattered scarce GFP+ donor cells were found in the meninges (subarachnoid space of the longitudinal fissure is shown) and choroid plexus of the Rag2^{-/-} brain. Insets show co-labeling for GFP and CD3, a pan T-cell marker. DAPI stains cell nuclei. Magnification bar = 10 μm.