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Inflammatory Th17 cells promote depression-like behavior in mice

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Abstract

Background—Recognition of substantial immune-neural interactions is revising dogmas about their insular actions and revealing that immune-neural interactions can substantially impact CNS functions. The inflammatory cytokine interleukin-6 promotes susceptibility to depression and drives production of inflammatory T helper 17 (Th17) T cells, raising the hypothesis that in mouse models Th17 cells promote susceptibility to depression-like behaviors.

Methods—Behavioral characteristics were measured in male mice administered Th17 cells, CD4⁺ cells, or vehicle, and in ROR γ T^{+/GFP} mice or male mice treated with ROR γ T inhibitor or anti-IL-17A antibodies.

Results—Mouse brain Th17 cells were elevated by learned helplessness and chronic restraint stress, two common depression-like models. Th17 cell administration promoted learned helplessness in 89% of mice in a paradigm where no vehicle-treated mice developed learned helplessness, and impaired novelty suppressed feeding and social interaction behaviors. Mice deficient in the ROR γ T transcription factor necessary for Th17 cell production exhibited resistance to learned helplessness, identifying modulation of ROR γ T as a potential intervention. Treatment with the ROR γ T inhibitor SR1001, or anti-IL-17A antibodies to abrogate Th17 cell function, reduced Th17-dependent learned helplessness.

Conclusions—These findings indicate that Th17 cells are increased in the brain during depression-like states, promote depression-like behaviors in mice, and specifically inhibiting the production or function of Th17 cells reduces vulnerability to depression-like behavior, suggesting antidepressant effects may be attained by targeting Th17 cells.

Keywords

antidepressant; depression; learned helplessness; neural-immune interactions; Th17 cells; RORgamma T

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Introduction

Major depression is a progressive, debilitating disease that afflicts nearly 20% of people in the United States (1), yet available antidepressants are often inadequate (2). Progress in developing new antidepressants is hindered by a lack of understanding of the causes of depression, disease heterogeneity due to the influence of numerous genetic and environmental factors differentially affecting symptoms, limited animal models of depression, and often a narrow focus in drug development on traditional neurotransmitter systems.

In spite of these difficulties, potential new therapeutic targets are being identified, including a growing recognition that immune system actions profoundly impact mood regulation (3–6). This has been particularly well-established for inflammatory signals from the innate immune system being linked to depression in human and rodent studies (7). For example, the inflammatory cytokine interleukin-6 (IL-6) is elevated in the serum of a subset of depressed patients, is activated in conditions associated with depression in humans and in rodent models, and is sufficient to induce depressive-like behavior in rodent models, whereas deletion of the IL-6 gene is protective (3–5, 8). However, although anti-inflammatory interventions have been reported to provide some antidepressant actions in a portion of patients, they have not been found to be widely efficacious antidepressants on their own, possibly because inflammation only contributes to depression in a portion of patients and because inflammation also alters the adaptive immune system. This is exemplified by IL-6, which drives the production of T helper 17 (Th17) cells, a subset of CD4⁺ T cells that is a major contributor to the pathogenicity of several inflammatory and autoimmune conditions (9–11).

Since inflammatory cytokines that direct T cell subtype production are altered in depressed patients (12–14), it is likely that depression is associated with changes in T cell subtypes. However, little is known about the impact of T cells on mood regulation and depression, although alterations in T cell characteristics have been reported in patients with major depression (15–18), there is some evidence that mood-related behaviors in rodents are influenced by T cells (19–22), and T cells have been shown to directly impact the brain. This has been particularly well-established for cognitive functions, such as the finding that immune deficiency in mice causes cognitive impairments that are reversed by replenishment of CD4⁺ T cells (23–27). Furthermore, CD4⁺ T cells are necessary for hippocampal neurogenesis in adult rodents, a process linked to some forms of learning (24, 28–30). These and other findings have established that maintenance of certain brain functions is dependent on T cell actions in the CNS (31, 32). In opposition to this, inflammatory subtypes of T cells, particularly Th17 cells, are clearly deleterious in diseases such as multiple sclerosis (9–11, 24). Thus, it appears that T cells subtype-selectively bolster particular neural functions in a healthy CNS, and that disease- or environment-induced shifts in the balance of T cell subtypes can switch their actions from beneficial to detrimental.

Altogether, it is evident that T cells can modify CNS functions, and that depression is associated with an environment capable of altering the population of subsets of CD4⁺ T cells, raising the possibility that altered subtypes of T cells may impact mood regulation and mood disorders. Because the conditions to promote production of Th17 cells are prevalent in depressed patients, and Th17 cells are deleterious to the CNS, we tested the hypotheses that Th17 cells may promote the induction of depression-like behavior in mice.

Methods and materials

Mice and behavioral assessments

C57BL/6 and C57BL/6–129 *Rorc*(γ T)^{+/GFP} (6–12 weeks) male mice were from the Jackson Laboratories and GSK3^{S21A/S21A/S9A/S9A} knockin mice were from Dr. Dario Alessi. 6 week old mice were used to prepared T cells while 8–12 week old mice were used for behavioral assessments. The *Rorc*(γ T)^{+/GFP} mice were obtained by crossing *Rorc*(γ T)^{+/GFP} × *Rorc*(γ T)^{+/GFP}, to produce 50% *Rorc*(γ T)^{+/GFP}, 25% wild-type, and 25% *Rorc*(γ T)^{GFP/GFP} mice, and behavioral experiments were carried out with littermates. Mice were housed in light and temperature controlled rooms and treated in accordance with NIH and university Institutional Animal Care and Use Committee regulations.

For the novelty-suppressed feeding (NSF) test (33), mice were weighed before and after food deprivation for 24 hr, and placed for 10 min in a brightly lit open field apparatus with a food pellet at the center on a slightly (1 cm) elevated platform. Behavior was monitored, and the latency to begin feeding was recorded. Upon returning to their home cage, the total amount of food consumed during a 5 min period was measured to test whether feeding differences in the novel environment were due to differences in hunger or motivation.

Learned helplessness was measured using a standard learned helplessness paradigm or a modified inescapable shock protocol, referred to as the reduced intensity inescapable shock protocol, and 24 hr later mice were tested with 30 escape trials as described previously (34). Mice were placed in one side of a Gemini Avoidance system shuttle box (San Diego Instruments, San Diego, CA, USA) with the gate between chambers closed. For standard learned helplessness, 180 inescapable foot shocks were delivered at an amplitude of 0.3 mA, a duration of 3–5 s per shock, and a randomized inter-shock interval of 5–45 s (35). In a modified inescapable shock protocol, referred to as the reduced intensity inescapable shock protocol, mice were given 180 foot shocks with amplitude of 0.3 mA and fixed 3 s shock duration, and a randomized inter-shock interval of 5–25 s (34). Twenty-four hours after inescapable foot shocks, mice were returned to the shuttle box and the escape task was tested by giving 30 escape trials with each trial preceded by a 0.3 mA foot shock for a maximum duration of 24 s. The door of the chamber opens at the beginning of the foot shock administration to allow mice to escape. Latency to escape the shock was recorded using Gemini software, and trials in which the mouse did not escape within the 24 s time limit were counted as escape failures. Mice with greater than 15 escape failures were defined as learned helpless. When indicated mice were injected i.p. once with 100 mg/kg lithium chloride as described (36) 24 h after the inescapable foot shocks. Mice used for learned helplessness were not used for other behavior measurements, whereas the same mice were used for the social interaction measurements and the NSF test. For chronic restraint stress, mice were placed individually in a 50 ml ventilated conical tube for 2 hrs daily for 2 weeks.

Th17 cell preparation and transfer

CD4⁺ T cells were isolated as described previously (37) and in the Supplementary Materials. CD4⁺ T cells cultured with splenic feeder cells were activated with 2.5 µg/mL of anti-CD3 (clone 145–11), and differentiated to Th17 cells by addition of IL-6 (20 ng/mL; Peprotech), TGFβ (5 ng/mL, Peprotech), anti-IL-4 (10 µg/mL; clone 11B11, UAB core facility) and anti-IFNγ (10 µg/mL; clone XMG1.2, UAB core facility). After 5 days of differentiation toward Th17 cells, cells were recovered after histopaque gradient purification, and resuspended in PBS. An aliquot of cells was used to evaluate the percent of Th17 cells (~40%) and ~10–20×10⁶ undifferentiated CD4⁺ T or Th17 cells were injected in 500 µL PBS by tail vein 48 h prior to behavioral testing. Where indicated, mice were injected intraperitoneally with 100 µg anti-IL-17A, or 125 µg SR1001 daily beginning 1 day before

Th17 cell transfer, and this was continued throughout the experiment. As controls, mice were injected i.v. with $\sim 10\text{--}20 \times 10^6$ CD4⁺ T cells to evaluate the effect of non-differentiated cells, or with 500 μL PBS to mimic the stress of tail vein i.v. injection 48 h prior to behavioral testing. Intracellular cytokine staining was carried out as described previously (37) and in the Supplementary Information.

Statistical analysis

Histograms represent Means $\pm$ SEM. Statistical significance was analyzed with a one-way multiple range analysis of variance (ANOVA) for multiple comparisons or with unpaired/paired t-test using Prism software. A p value <0.05 was considered significant.

Results

Stress is a critical factor in promoting susceptibility to depression (2, 38), and is modeled in rodents with the learned helplessness paradigm of depression-like behavior (39) to assess modulators of mood, while limitations of animal models are also well-recognized (38, 40, 41). The traditional learned helplessness paradigm involves stressing mice with mild, unpredictable and inescapable foot shocks (IES), and typically 24 hr later exposing mice to mild foot shocks from which they are free to escape. Due to the initial stressful period, many mice fail to escape even when it is readily available, and learned helplessness is defined as failure to escape in >15 out of 30 escapable trials. Learned helplessness is also advantageous to many models of depression-like behavior because the parameters can be adjusted to measure either resistance to learned helplessness using the traditional protocol, or increased susceptibility to learned helplessness using diminished aversive stimuli (34).

Because the conditions to promote differentiation of Th17 cells are present in depressed patients, and Th17 cells are deleterious to the CNS, we tested if learned helplessness resulted in increased brain Th17 cells in wild-type mice and if this was blocked by administration of the mood stabilizer and glycogen synthase kinase-3 (GSK3) inhibitor lithium, which has an antidepressant effect in the learned helplessness paradigm (36). After exposure to IES, the subsequent escapable test was used to separate mice into two cohorts, those that exhibited learned helplessness and those that escaped (Figure 1A). Immediately after the escapable shock session, cells isolated from the brain were analyzed by FACS. The number of Th17 cells, which are identified by positive co-staining for CD4⁺ and for IL-17A (i.e., IL-17A⁺ CD4⁺ T cells) was increased by 78% in the learned helplessness mouse brains compared to the non-depressed mice, even though all mice were exposed to the same foot shock protocol. A 27% increase in brain Th1 cells, which are identified by positive co-staining for CD4⁺ and IFN γ (IFN γ ⁺ CD4⁺ T cells), was found in learned helpless mice, whereas there was no difference between groups in T regulatory cells (Tregs; FoxP3⁺ CD4⁺ T cells) (Figure 1B). Lithium pretreatment reduced the percent of mice meeting learned helplessness criteria from 60% to 10%, and blocked the increases in brain Th17 and Th1 cells, consistent with a previous report that lithium reduces the production of Th17 cells in mice in vivo (42). There were no significant differences among these groups in the total numbers in brain of CD4⁺ T cells, CD8⁺ T cells, or activated microglia (identified by staining for F4/80⁺CD45^{int} cells) (Figure 1C). Furthermore, there were no differences in the number of brain CD11c⁺CD45^{high} dendritic cells (DCs) or F4/80⁺CD45^{high} cells (activated macrophages) (Figure 1C), indicating there was no difference in blood brain barrier permeability between mice that were or were not learned helpless, which would be evident by an increase in the number of infiltrated DCs and macrophages. The numbers of Th17, but not Th1 cells or Tregs, also were greater in the spleens of learned helpless mice than in nonlearned helpless mice or in mice treated with lithium (Figure 1D).

To test if brain Th17 and Th1 cells increased in another model of depression, mice were subjected to restraint stress for two weeks. This is widely used to induce a depressive phenotype (33), substantiated by a 15% lowered body weight (Supplemental Figure S1), a marker of chronic stress. As with learned helplessness, chronic restraint stress resulted in increased brain levels of Th17 cells (Figure 1E) and Th1 cells (Figure 1F). Taken together, these results suggest that increased Th17 or Th1 cells in the brain may contribute to, or be an outcome of, depression-like behavior.

Genetically predisposed individuals exposed to environmental factors, such as stress, may be especially vulnerable to major depression. Glycogen synthase kinase 3 (GSK3) knockin mice possess a genetic predisposition that increases deleterious responses to stress, which is evident upon exposure to mild aversive stimuli (34). GSK3 knockin mice express normal levels of both GSK3 isoforms, GSK3 α and GSK3 β , but with serine-to-alanine mutations, S9A-GSK3 β and S21A-GSK3 α , to maintain GSK3 maximally active, since it cannot be inhibited by serinephosphorylation (43, 44). Exposure to only escapable foot shocks, without a prior session of inescapable foot shocks, causes learned helplessness behavior in a portion of GSK3 knockin mice, but not wild-type mice (34). A single session of only escapable foot shocks induced learned helplessness in ~40% of GSK3 knockin mice (Figure 1G). Comparing the two cohorts of GSK3 knockin mice that received the same foot shock protocol revealed that the numbers of Th17 and Th1 cells were significantly higher in the brains of GSK3 knockin mice that failed to escape than in those that escaped, while the numbers of macrophages and DCs were the same in both groups (Figure 1H). Thus, conditions causing depression-like behavior consistently increased mouse brain Th17 and Th1 cells.

The well-established pathological actions of Th17 cells in other diseases (45–47) and their increases in the brains and spleens of learned helpless and stressed mice raised the possibility that Th17 cells may promote susceptibility to depression-like behavior. Therefore, we tested if administration of Th17 cells to wild-type mice was sufficient to promote depression-like behaviors, including learned helplessness, impaired novelty-suppressed feeding (NSF), and impaired social interactions. Th17-recipient mice did not differ from vehicle-treated controls in locomotor activity in a novel open field (Supplemental Figure S2A) or pain sensitivity (Supplemental Figure S2B). Mice were subjected to inescapable foot shocks of reduced intensity that did not induce learned helplessness in vehicle- or undifferentiated CD4⁺ T cell-treated control mice, whereas 89% of Th17-recipient mice met the learned helplessness criteria of >15 failures out of 30 trials (Figure 2A). The latency time to escape demonstrated rapid escape times by control mice, whereas Th17 recipient mice consistently failed to escape (Figure 2B). The Th17-recipient mice contained elevated brain levels of IL-17A⁺ CD4⁺ T cells (Th17 cells), but no differences from control mice in IFN γ ⁺ CD4⁺ T cells (Th1 cells), FoxP3⁺ CD4⁺ T cells (Tregs), total CD4⁺ T cells, CD8⁺ T cells, F4/80⁺CD45^{int} cells (activated microglia), F4/80⁺CD45^{high} cells (activated macrophages), and CD11c⁺CD45^{high} cells (DCs) (Figure 2C), confirming that Th17 cells increase in the brain of learned helpless mice. The spleens of Th17 recipient mice also contained elevated levels of IL-17A⁺ CD4⁺ T cells, and a slight but statistically significant increase in total CD4⁺ T cells, but no changes of other cell types (Supplemental Figure 3), confirming that sufficient injected cells were viable after administration to increase the overall populations of CD4⁺ and Th17 cells in the spleen. These results demonstrate that elevation of Th17 cells is sufficient to increase susceptibility to the learned helplessness model of depression-like behavior.

Administration of Th17 cells also affected behavior in the NSF test and in social interaction. In the NSF test, Th17-recipient mice displayed significantly longer latencies to feed when placed in a novel arena with food following 24 hr food deprivation compared with control

CD4⁺ T cell-treated mice (Figure 2D). This was not due to suppressed appetite and there was no difference in weight loss between groups (Supplemental Figure S3B). Although Th17-recipient mice did not exhibit overt changes in sociability as estimated by time spent in the chamber with a novel conspecific mouse, reduced nose contacts indicated diminished social interaction (Figure 2E). Thus, administration of Th17 cells was sufficient to impair several behaviors that have been associated with depression.

These results raised the possibility that reducing Th17 cells may provide a feasible intervention to bolster resistance to depressive-like conditions. Th17 cells are absent in mice with depleted retinoid-related orphan receptor- γ T (ROR γ T), a transcription factor that drives the differentiation of Th17 cells that is thought to be expressed almost exclusively in Th17 cells (48), and is a widely used marker of Th17 cells (49). ROR γ T knockout mice exhibited strong resistance to learned helplessness (Supplemental Figure S4A), consistent with the postulate that Th17 cells promote this behavior. However, examination of the immune system of these mice revealed multiple alterations (48), Supplemental Figure S4B). Therefore, we examined heterozygote Rorc(γ T)^{+/-GFP} mice, which produce a greatly diminished Th17 cell population because of the reduction of ROR γ T expression (48), as we recently confirmed (37). Rorc(γ T)^{+/-GFP} mice did not differ from littermate wild-type mice in locomotor activity in a novel open field (Figure 3A). To test for an anti-depressant effect of reduced Th17 cells, mice were exposed to IES, and 24 hr later, the escapable shock test was used to identify mice exhibiting learned helplessness. Rorc(γ T)^{+/-GFP} mice exhibited a strong resistance to the learned helplessness paradigm, with only 25% of mice meeting the learned helplessness criteria, compared with 75% of littermate wild-type mice (Figure 3B). The latency time to escape also demonstrated that the wild-type mice maintained learned helpless behavior throughout the test in contrast to the Rorc(γ T)^{+/-GFP} mice (Figure 3C).

Evaluation of cells in the brains of mice after the learned helplessness paradigm confirmed that, compared with wild-type mice, Rorc(γ T)^{+/-GFP} mice had lower levels of IL-17A⁺ CD4⁺ T cells (Th17 cells), a slight but not statistically significant increase in IFN γ ⁺ CD4⁺ T cells (Th1 cells), no difference in FoxP3⁺ CD4⁺ T cells (Tregs), and lower total CD4⁺ T cells (Figure 3D). Rorc(γ T)^{+/-GFP} mouse brain CD8⁺ T cells and F4/80⁺CD45^{int} cells (activated microglia) did not differ from controls, but F4/80⁺CD45^{high} cells (activated macrophages) and CD11c⁺CD45^{high} cells (DCs) were lower in Rorc(γ T)^{+/-GFP} mice than wild-type mice (Figure 3E). Thus, Rorc(γ T)^{+/-GFP} mice displayed a remarkable resistance to learned helplessness behavior in this paradigm that is robustly effective in wild-type mice, further indicating that reduction of Th17 cells increases resistance to learned helplessness behavior.

Two approaches were used to test the feasibility of pharmacologically reducing the pathogenicity of transferred Th17 cells as a therapeutic intervention for depression. Using the model of Th17 cell administration, mice were treated with anti-IL-17A antibodies to neutralize actions of Th17 cells, and to reduce the differentiation of Th17 cells mice were treated with SR1001, a recently identified ROR γ T inhibitor (50). Anti-IL17A therapy and inhibition of ROR γ T did not alter locomotor activity (Figure 4A), but each treatment significantly reduced learned helplessness and reduced the latency time to escape (Figure 4B,C). Measurements of Th17 cells in the spleen and brain demonstrated a significant reduction of Th17 cells after SR1001 treatment (Supplemental Figure S5). These findings confirmed that blocking Th17 actions provide antidepressant effects in Th17-dependent learned helplessness. Thus, brain Th17 cells increase with depressive-like behavior, and intervening at the level of IL-17A or ROR γ T is sufficient to neutralize the Th17-dependent depression-like behavior effect.

Discussion

The growing evidence of causative links between inflammation and depression (3, 4) indicates that targets of inflammatory molecules contribute to establishing a pro-depressant environment. This study focused on T cells because they are major sensors of the cytokine environment, each subtype of T helper CD4⁺ cell responds to a different cytokine signature, and, importantly, T cells are more enduring than transiently expressed cytokines, potentially enabling T cells to contribute to the prolonged and recurrent episodes characteristic of major depression. IL-6 is an inflammatory cytokine with well-established links to depression, stress induces IL-6 expression, and IL-6 is a crucial driver of Th17 cells that are detrimental to the CNS, raising the possibility that Th17 cells contribute to mediating the depressive outcome of excessive inflammation. We found that Th17 cells accumulate in the brains of mice subjected to two models of depression-like behavior, administration of Th17 cells promoted three behavioral models of depression, and reducing Th17 cells provided resistance to learned helplessness. This extends to mood regulation previous findings that T cells are present and functional in the brain (32) where they modulate higher functions, such as cognition.

The initial direct evidence that Th17 cells may promote depression-like behavior was the increased Th17 cell numbers found in mouse brain after depression-inducing stimuli, including a 78% increase of Th17 cells in the brains of mice that displayed learned helplessness. Importantly, in this paradigm all mice received the same aversive stimuli, which is well-established to produce learned helplessness in only a portion of treated mice. Thus, despite matching treatments, only those mice with increased Th17 cells displayed learned helplessness, raising the possibility that Th17 cells may contribute to the development of depression-like behavior. Th1 cells also may play a regulatory role since they increased in mouse brains after depression-inducing stimuli, and these associated increases in Th17 and Th1 cells may be linked to recent evidence that Th1 cells can be derived from Th17 cells (51). In contrast, anti-inflammatory Treg levels were not altered in these conditions, suggesting that proinflammatory cytokines are driving the pathogenic T cells.

A causative role for Th17 cells in depression-like behaviors is supported by the findings that altering Th17 cells in mice modified the behavioral outcomes. Administration of Th17 cells was used to test if pre-existing elevated Th17 cells promoted learned helplessness by taking advantage of the fact that untreated wild-type mice seldom develop learned helplessness after subjection to a mild foot shock paradigm. Remarkably, 89% of mice developed learned helplessness after receiving Th17 cells two days previously, a time that allows for recovery from the acute treatment and proliferation of administered T cells. Th17-recipient mice also displayed deficits in NSF and social interactions, two other behaviors that have been linked to a depressive phenotype. Thus, the adaptive immune system, in particular the Th17 cell burden, profoundly affects stress-induced behavioral responses that may model mood dysregulation. However, this study was for the most part limited to evaluations of the learned helplessness model, so it remains to be determined if Th17 cells also promote other models of depression-like behavior in rodents and major depression in humans.

Methods capable of reducing Th17 cell levels in vivo are limited, with ROR γ T knockout mice being the most widely used approach (48, 49). Although ROR γ T is a characteristic marker of Th17 cells, since it may have unknown additional functions in other cell types, results with ROR γ T knockout mice must be considered in conjunction with other means of modulating Th17 cells, such as antibodies that block the actions of IL-17A or recently described ROR γ T inhibitors that limit differentiation of Th17 cells (50). Indeed, we found significant immune alterations in ROR γ T knockout mice, which led us to focus on

Rorc(γ T)^{+/GFP} mice with only partial depletion of ROR γ T. Although in these mice Th17 cells were only partially depleted, not abolished, the mice nevertheless displayed a robust 3-fold increase in resistance to acquisition of learned helplessness, with 75% of the Rorc(γ T)^{+/GFP} mice resistant to a paradigm in which only 25% of wild-type mice were resistant. Thus, even partial reduction of Th17 cell production provides significant protection from the vulnerability of mice to learned helplessness. It is also notable that the mood stabilizer lithium both blocked learned helplessness and Th17 cell accumulation in the brain, which may be due to the previously identified action of GSK3 inhibitors to block Th17 cell production (37). Moreover, inhibition of ROR γ T confirmed that pharmacological intervention aimed at Th17 cell production provides resistance to Th17 cell-dependent depressive-like behavior, providing a potential new avenue of intervention. The antidepressant effect of anti-IL-17A therapy demonstrated that IL-17A is a critical cytokine mediating Th17-dependent learned helplessness, in accordance with its detrimental effects in autoimmune diseases, although it remains to be determined if this results from peripheral actions or effects on cells in the CNS, such as its previously identified actions on astrocytes that may alter astrocyteneuronal interactions (52).

These findings indicate for the first time a significant contribution of Th17 cells to promote susceptibility to depressive-like behavior. Th17 cells are likely only one factor among many that modify susceptibility to depression-like behavior. Thus, interfering with the production or actions of Th17 cells may prove most effective in patients with depression that are pre-identified as having elevated levels of IL-6, which drives Th17 cell production, or of IL-17A, which is produced by Th17 cells. These findings demonstrate that the adaptive immune system significantly impacts susceptibility to learned helplessness and other behaviors that may be relevant to mood regulation, suggesting that interventions being developed for suppressing Th17 cells in autoimmune diseases may provide a starting point to develop new treatments for mood disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Th17 cells accumulate in the brains of mice exhibiting learned helplessness. Wild-type mice were subjected to mild inescapable foot shocks, 24 h later lithium chloride (LiCl; 100 mg/kg; i.p.) was administered, and after 24 h mice were tested with escapable foot shocks. (A) Escape failures after standard IES. Each symbol represents total escape failures in 30 trials for an individual mouse, bars represent group means $\pm$ sem ($n=10-21$ mice/group, t -test $*p<0.05$, $t=3.030$, $df=31$). Mice were separated into 3 groups: mice that failed to escape <15 of 30 trials (nondepressed, ND), mice that failed to escape >15 of 30 trials (depressed, D), and non-depressed lithium-treated mice. After behavior measurements, the indicated (B) Th

cells or **(C)** immune cells from the brain, or **(D)** Th cells from the spleen, were stained and analyzed by flow cytometry. Bars represent mean $\pm$ sem. (ANOVA, * $p < 0.05$ compared with values from non-depressed mice, $df=10$, $F=8.466$ (Th17 cells), * $p < 0.05$ compared to ND +Li, $df=7$, $F=9.614$ (Th1 cells)). **(E)** Exposure to chronic restraint stress (CS) for two weeks increased the number of Th17 cells and **(F)** Th1 cells in the brains of mice compared to non-stressed mice ($n=5$ /group, Mann-Whitney test * $p < 0.05$, † $p=0.06$). **(G)** GSK3 knockin (KI) mice received escapable shocks only, and 46% of the mice failed to escape >15 of 30 trials (depressed, D). Each symbol represents total escape failures in 30 trials for an individual mouse ($n=13$). Bars in panels A–G are representative of 3 independent experiments. **(H)** After behavior measurements, Th cells recovered from 5 brains per group (ND and D) of GSK3 knockin mice were pooled together, stained as indicated, and analyzed by flow cytometry.

Figure 2.

Transfer of Th17 cells increased susceptibility to depression-like behavior. Wild-type mice received either PBS, Th17 cells, or CD4⁺ T cells, 48 h later mice were subjected to reduced-intensity inescapable foot shocks that did not induce learned helplessness in vehicle- or CD4⁺ T cell-treated mice, and 24 h later mice were tested with escapable foot shocks. **(A)** Escape failures after reduced intensity IES. Each symbol represents total escape failures in 30 trials for an individual mouse, bars represent group means $\pm$ sem (n=3–9 mice/group, ANOVA * p <0.05, df =20, F =58.67). **(B)** Average escape latency during 5-trial blocks after reduced intensity IES. WT mice injected with PBS or CD4⁺ cells had an average of 5.5 ± 1.4 or 4 ± 0.7 sec escape latency, respectively, whereas mice injected with Th17 cells had an average of 22.7 ± 0.8 sec escape latency. Bars represent group means $\pm$ sem (n=3–9 mice/group, ANOVA * p <0.05, df =17, F =98.30). After behavior measurements, the indicated **(C)** Th cells or immune cells were stained and analyzed by flow cytometry. Bars represent mean $\pm$ sem (n=3–9 mice/group ANOVA * p <0.05 compared with PBS-treated mice, df =21, F =10.84). **(D)** In the NSF test, Th17-recipient mice displayed significantly longer latencies to feed when placed in a novel arena with food following 24 hr food deprivation compared with control CD4⁺ T cell-treated mice (n=4 mice/group, * p <0.05 compared with CD4-treated mice, paired t-test, df =3, t =3.200). **(E)** Th17-recipient mice demonstrated significantly fewer nose contacts with a novel conspecific mouse than did CD4⁺ T cell-treated mice but did not differ in the time spent in each chamber of the apparatus (CH1, chamber 1 containing the conspecific mouse; CH2, empty middle chamber 2; CH3, chamber 3, equivalent to CH1 without a mouse) (n=5 mice/group, * p <0.05 compared with CD4-treated mice, paired t-test, df =4, t =3.670).

Figure 3.

Depletion of Th17 cells in *Rorc*(γT)^{+/GFP} mice reduces acquisition of learned helplessness. (A) Basal activity of wild-type or *Rorc*(γT)^{+/GFP} mice was measured in an open field for 30 min. (B–D) Mice were subjected to standard IES, and 24 h later tested with escapable foot shocks. (B) Escape failures after standard IES. Each symbol represents total escape failures in 30 trials for an individual mouse, bars represent group means (n=8–12 mice/group, t-test *p<0.05, df=18, t=2.267). (C) Average escape latency during 5-trial blocks after standard IES. WT and *Rorc*(γT)^{+/GFP} mice had an average of 19.4±0.4 and 10.9±1.3 sec escape latency, respectively. (n=8–12 mice/group, Mann Whitney *p<0.05). After behavior measurements, the indicated (D) Th cells or immune cells were stained and analyzed by flow cytometry. Bars represent mean ± sem. (n=8–12 mice/group Mann Whitney *p<0.05).

Figure 4.

Inhibiting ROR γ T or IL-17A has antidepressant effects in Th17-dependent learned helplessness. Wild-type mice pretreated where indicated with anti-IL-17A antibodies or SR1001, a ROR γ T inhibitor, received Th17 cells. **(A–B)** Mice were subjected to reduced intensity IES, and 24 h later tested with escapable foot shocks. **(A)** Escape failures after reduced IES. Each symbol represents total escape failures in 30 trials for an individual mouse, bars represent group means ($n=8-10$ mice/group, ANOVA $*p<0.05$ compared with untreated Th17 recipient mice, $df=45$, $F=8.220$). **(B)** Average escape latency during 5-trial blocks after reduced intensity IES. Mice receiving Th17, Th17+ anti-IL17A, and Th17 + SR1001 mice had an average of 21.8 ± 1.6 , 13.6 ± 0.6 and 15.1 ± 0.8 sec escape latency, respectively. ($n=8-10$ mice/group, ANOVA $*p<0.05$ compared with untreated Th17 recipient mice, $df=17$, $F=15.77$).