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CD4-Positive T Lymphocytes Provide a Neuroimmunological Link in the Control of Adult Hippocampal Neurogenesis¹

Susanne A. Wolf,^{2*†} Barbara Steiner,^{2*‡} Akgul Akpinarli,[§] Thomas Kammertoens,^{¶||} Christina Nassenstein,[#] Armin Braun,[#] Thomas Blankenstein,^{||} and Gerd Kempermann^{3* **}

Adult hippocampal neurogenesis occurs in an exceptional permissive microenvironment. Neuroimmunological mechanisms might be prominently involved in the endogenous homeostatic principles that control baseline levels of adult neurogenesis. We show in this study that this homeostasis is partially dependent on CD4-positive T lymphocytes. Systemic depletion of CD4-positive T lymphocytes led to significantly reduced hippocampal neurogenesis, impaired reversal learning in the Morris water maze, and decreased brain-derived neurotrophic factor expression in the brain. No such effect of CD8 or B cells was observed. Repopulation of RAG2^{-/-} mice with CD4, but not with CD8 cells again increased precursor cell proliferation. The T cells in our experiments were non-CNS specific and rarely detectable in the healthy brain. Thus, we can exclude cell-cell contacts between immune and brain cells or lymphocyte infiltration into the CNS as a prerequisite for an effect of CD4-T cells on neurogenesis. We propose that systemic CD4-T cell activity is required for maintaining cellular plasticity in the adult hippocampus and represents an evolutionary relevant communication route for the brain to respond to environmental changes. *The Journal of Immunology*, 2009, 182: 3979–3984.

The immune and the nervous system are interconnected (1, 2) and share intriguing common features at the molecular level (3). This cross-talk is increasingly acknowledged as relevant for higher cognitive functions, most likely including events of plasticity (4, 5). The most prominent example of cell-based plasticity is adult neurogenesis and its activity-dependent control (6–8).

The dentate gyrus (DG)⁴ of the hippocampus is closely linked to learning and memory consolidation, and is one of the two regions in the adult brain in which neurogenesis occurs throughout life. The exact functional relevance of the newborn neurons in the DG is not yet fully understood (9–11), but several lines of evidence point to a contribution of the new neurons to long-term adaptation (12, 13). Physiological and certain learning stimuli robustly induce adult hippocampal neurogenesis (14–17). Whereas most studies have concentrated on extrinsic mechanisms that affect the neuropermissive microenvironment, only few endogenous regulators

have been described to date. One key factor for creating neurogenic permissiveness might be the immune system.

Microglial activation led to reduced neurogenesis in the DG, an effect that could be reversed by anti-inflammatory treatment (18, 19). However, any possible role for components of the adaptive immune response including B and T lymphocytes in the control of adult neurogenesis under healthy conditions has not yet been investigated.

Ziv et al. (20) reported that, in immune-deficient mice, hippocampal neurogenesis was markedly impaired and could not be enhanced by environmental enrichment, but was restored and boosted by T cells recognizing a specific CNS Ag. In addition, they investigated the role of CNS-specific T cells for spatial learning and memory performance using transgenic mice expressing a TCR specific for myelin basic protein. Under physiological conditions only few T cells are found in the brain, although T cell trafficking to the brain does occur constantly. No or only few myelin basic protein-specific T cells are circulating at a given time point (21). We therefore assumed that nonencephalitogenic cell-based immunological mediators in the control of adult hippocampal neurogenesis might exist.

In models of general immune deficiency, impairment in cognitive function has been shown (22), and Bryniskikh et al. (23) described a role for T cells in cognition and behavior. In neither case, however, a potential relation to adult neurogenesis has been addressed.

We therefore depleted specific cell subsets of the adaptive immune system and used genetically modified animals that lack either T or B cells or both as well as CD4 and common γ -chain knockout (KO) animals. We measured baseline neurogenesis levels and the response to physical activity. Because T cells invading the CNS release brain-derived neurotrophic factor (BDNF) and can activate BDNF release from neural bystander cells (24, 25), we measured BDNF levels in the brain. Cognitive performance was assessed in the Morris water maze (MWM) (26).

Materials and Methods

Animals

B cell-deficient (μ MT) mice were generated and bred in the animal facility of the Max Delbrück Center, as described elsewhere (27). The animal study was approved by Landesamt für Gesundheit und Technische Sicherheit Berlin.

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⁴ Abbreviations used in this paper: DG, dentate gyrus; BDNF, brain-derived neurotrophic factor; DCX, doublecortin; KO, knockout; MWM, Morris water maze; NGF, nerve growth factor.

RAG1^{-/-} mice on the C57BL/6 background that lack T and B cell function were bred in the animal facility of the Max Delbrück Center and originally obtained from Charles River Laboratories. RAG1^{+/-} males and females were crossed to obtain RAG1^{-/-} and RAG1^{+/-} littermates.

RAG2^{-/-} mice on the C57BL/6 background that lack T and B cell function were bred in the animal facility of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

CD4^{-/-} mice (28) were bred at the animal facility of Charité University Medicine.

Common γ -chain KO C57BL/6J $\times$ C57BL/10SgSnAi-(KO) common γ -(KO)Rag2 were obtained from Taconic Farms and were originally made at National Institutes of Health, National Institute of Allergy and Infectious Diseases. The common γ KO mouse lacks functional receptors for many cytokines, including IL-2, IL-4, IL-7, IL-9, and IL-15. As a consequence, lymphocyte development is greatly compromised. The mouse lacks NK cells and produces only a small number of T and B cells. To eliminate the residual T and B cells, this mouse was crossed to the Rag-2-deficient mouse (29).

Animals were injected with 50 mg/kg BrdU (Sigma-Aldrich) in 100 μ l of 0.9% NaCl once for proliferation analysis 24 h after injection and three times at 3 consecutive days for survival analysis 4 wk after the last injection.

Depletion of lymphocyte subsets by specific Abs

Mice received 250 μ g of nonmitogenic (F(ab')₂, fragments) anti-CD3 (R&D Systems), 2 mg of anti-CD4 (GK 1.5; American Type Culture Collection), or anti-CD8 (2.43; American Type Culture Collection) mAb 2 days before BrdU injection. Armenian hamster IgG was used as control (R&D Systems), depletion lasting over 7 days (27). Specific depletion was confirmed using the FITC-RM-4-4 (anti-CD4; BD Pharmingen) and PE-53-5.6.7 (anti-CD8; BD Pharmingen) to analyze PBL after T cell depletion. Nondepleted C47BL/6 mice were used as controls. Cells were labeled and analyzed on a FACSCalibur with CellQuest software (BD Biosciences).

Repopulation of RAG2^{-/-} animals with T cell subsets

CD4- and CD8-positive cells were sorted out of lymph nodes and spleen of a C57BL/6 mouse using MACS, according to the manufacturer's protocol. For positive selection, we used CD4 (L3T4) and CD8 (Ly-2) Micro beads (Miltenyi Biotec). We injected into female RAG2^{-/-} mice i.p. a total of 2×10^6 CD4 or CD8 cells in 300 μ l of 0.9% NaCl per animal and assayed them for neurogenesis 4 days later. Control mice received 0.9% NaCl only. Due to regulations by the National Institutes of Health animal facility where the animals were housed, we were not able to use BrdU as a proliferation marker. Instead we used the intrinsic mitotic marker Ki67 (NovoCastra).

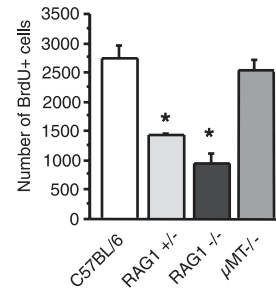
ELISA

For ELISA for nerve growth factor (NGF) and BDNF (R&D Systems), brains were homogenized in diluting reagent included in the ELISA kit. The plates were precoated with Ab to bind either NGF or BDNF. Triplicates of 200 μ l of each sample were incubated for 2 h. A standard dilution was made with NGF or BDNF provided in the kit. After washing, incubation with a biotinylated secondary Ab was performed, followed by incubation with streptavidin and a color reagent. The plate was read with an ELISA reader, and the results were normalized by brain weight. The data are presented as NGF and BDNF pg/mg brain tissue.

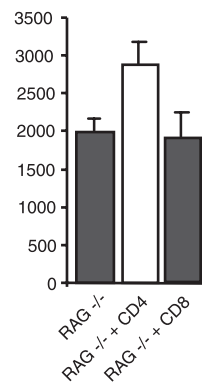
Immunohistochemistry, immunofluorescence, and quantification of BrdU-positive cells

Animals were perfused and brains were dissected from the skulls, and either the whole brain or one hemisphere (in the cases of the subsequent measurement of NGF and BDNF) was postfixed and cut, followed by immunohistochemistry and BrdU-positive cell quantification and phenotyping, as described previously (30). The primary Abs were applied in the following concentrations: rat anti-BrdU (1:500; Harlan Seralab), mouse anti-CD3 (1:100; BD Pharmingen), goat anti-doublecortin (DCX; 1:200; Santa Cruz Biotechnology), mouse anti-NeuN (1:100; Chemicon International), rabbit anti-Iba-1 (1:1000; Chemicon International), and rabbit anti-Ki67 (1:500; Abcam). The secondary Abs were as follows: anti-rat biotinylated (for BrdU), anti-mouse biotinylated (for CD3), anti-rabbit biotinylated (for Ki67), anti-rat RhodX, anti-goat FITC, anti-rabbit FITC, anti-mouse FITC, anti-rabbit Cy5, and anti-goat Cy5 (all 1:250; Dianova). For light microscopy we used the ABC kit (Vectastain; Vector Laboratories) and diaminobenzidine (Sigma-Aldrich) to visualize the positive cells. Double- and triple-labeled cells were analyzed and quantified by confocal microscopy, as described elsewhere (31).

A T cell depletion results in reduced number of BrdU-labeled cells in the dentate gyrus



B CD4 reconstitution of RAG1^{-/-} mice increases cell proliferation



C CD4 reconstitution of RAG1^{-/-} mice increases proliferating DCX cells

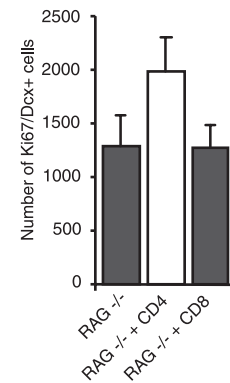


FIGURE 1. Increased hippocampal neurogenesis significantly depends on the presence of CD4-positive T cells. *A*, Significant decrease in the numbers of BrdU-positive cells in RAG1^{-/-} mice vs RAG1^{+/-} and C57BL/6 vs RAG1^{+/-} mice. In addition, we found no difference in cell proliferation between μ MT mice and healthy controls. *B* and *C*, Reconstitution of RAG2^{-/-} mice with CD4 T lymphocytes increased the number of Ki67-positive cells (*B*) and DCX/Ki67-positive cells (*C*). RAG2^{-/-} and RAG1^{-/-} mice differ in their baseline proliferation level.

Statistical analysis

All numerical analysis was performed using Statview 5.0.1 for Macintosh. For all comparisons, ANOVA was performed by Fisher's post hoc test when appropriate. Differences were considered statistically significant at $p < 0.05$.

Morris water maze

The mice were injected with anti-CD4 Ab 1 day before they were trained in the MWM and again on day 3 of the acquisition phase. The successful CD4 depletion was assessed by FACS analysis.

The MWM test is widely used to test rodents for spatial memory performance (26) and has been used by our group in previous studies. We followed the protocol devised by Wolfer et al. (32). Briefly, six trials of training, each maximally lasting for 2 min, were given each day. Latencies to reach the platform and swim paths were recorded with an automatic video tracking system (Ethovision; Noldus Information Technology).

Animals were exposed to the water maze that contained an escape platform submerged 1 cm below the water line. The platform was kept at a constant location within the pool during the first 3 days of training. The probe trial was conducted on the morning of the fourth day without the platform. After the probe trial, the invisible platform was placed in the quadrant opposite to the former (target quadrant) to start the reversal learning task for 2 more days. To control for parameters that are not hippocampus dependent, such as vision impairments, the task was afterward repeated with a visible platform. To evaluate learning of the spatial location of the platform, latencies to reach the platform (in seconds)/total length of swim path (in pixels converted to cm) were compared between trials. Additionally, the time spent in the target quadrant on the probe trials was used as an indicator of targeted searching for the platform. During the reversal learning, time spent in quadrant 1 (location of the platform during initial

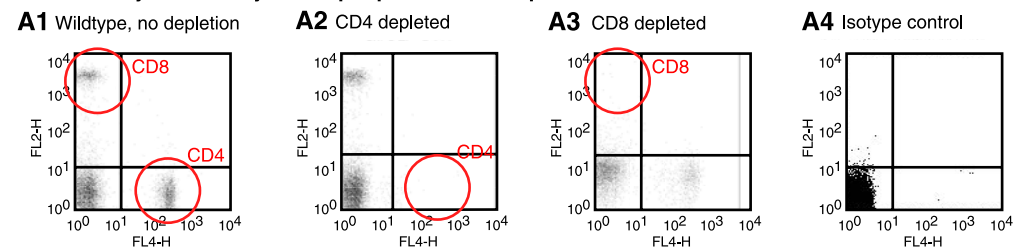
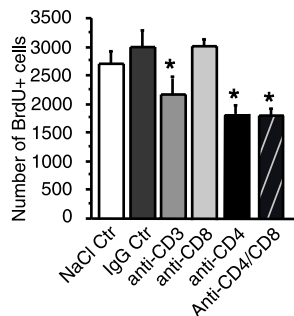
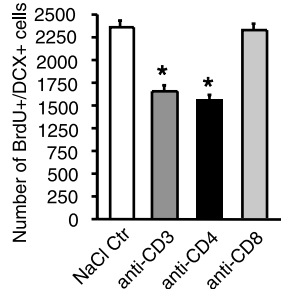
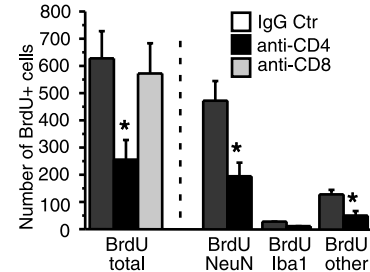
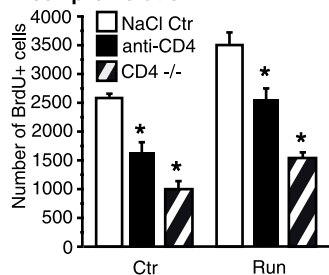
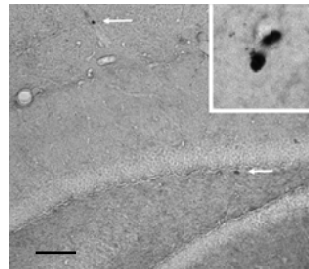
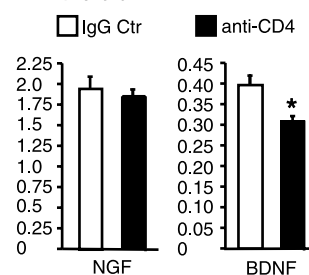
A Efficiency of antibody-based peripheral T cell depletion**B Effect of peripheral T cell depletion on cell proliferation****C Effect of peripheral T cell depletion on proliferation of DCX-positive cells****D Effect of peripheral T cell depletion on neuronal differentiation****E Effect of T cell depletion on exercise-induced cell proliferation****F The hippocampal dentate gyrus is essentially devoid of T cells****G Depletion of CD4+ cells decreases BDNF production in the brain**

FIGURE 2. Depletion of CD4-positive T cells leads to significantly impaired neurogenesis in the DG. *A*, FACS: Sufficient depletion of CD4-positive (A2) or CD8-positive (A3) T cells vs wild-type animals (A1). A4, Isotype control without T cells. *B*, Depletion of CD3-positive T cells with anti-CD3 Ab significantly decreased numbers of BrdU-positive cells in the DG. This effect could be confirmed by Ab-induced blockage of CD4-positive, but not CD8-positive T cells. *C*, Depletion of CD4-positive T cells significantly reduced the numbers of newborn DCX-positive cells. *D*, Anti-CD4 treatment led to a significant decrease in BrdU-positive cells and a significant decrease in the number of new NeuN-positive cells compared with IgG-treated controls. Significantly reduced numbers of BrdU-positive cells of undetermined phenotype (BrdU other) in anti-CD4-treated vs control animals. No differences were found in anti-CD8-treated animals vs controls. *E*, Running increased cell proliferation in the DG of healthy control mice. CD4 depletion resulted in reduction of the numbers of BrdU-positive cells. Both types of CD4-depleted mice responded to physical activity. *F*, Only few CD3-positive lymphocytes can be found in the DG (arrows). Typically, lymphocytes are found in proximity to blood vessels, here in the cortex, and sometimes as pairs (inset). Scale bar, 150 μ m (30 μ m for inset). *G*, Decreased BDNF production in brains of CD4-depleted animals vs IgG-treated controls. NGF production was not affected by CD4 depletion.

training) vs quadrant 3 (location of the platform during reversal training) was measured.

To analyze performance in the MWM test, we performed a repeated measure ANOVA test of the daily means. Analysis of the differences between the groups in the parameters escape latency, and distance moved per day, and the parameters for the trial session was done using the Fisher post hoc test, if applicable.

Results

Depletion of both T and B lymphocytes, but not B lymphocytes alone, reduces the number of proliferating cells in the DG

To investigate the role of specific cellular components of the adaptive immune response, we first compared RAG1^{-/-} mice that lack both, mature functional T and B cells and their RAG1^{+/-} littermates. RAG1^{-/-} mice showed lower baseline cell proliferation identified by BrdU staining. When we compared RAG1^{+/-} heterozygote animals with C57BL/6 WT animals, we could detect a

baseline difference in precursor cell proliferation (RAG1^{+/-} vs RAG1^{+/+}, $p = 0.002$; RAG1^{+/+} vs C57BL/6, $p = 0.0001$; ANOVA, $p < 0.0001$; $F_{2, 10} = 37.65$; Fig. 1A). Whether this implicates any functional difference between the heterozygotes and WT mice solely based on the lack of one RAG1 gene copy could not be differentiated in this study.

To distinguish between the role of T and B cells in the control of cell proliferation in the subgranular zone, we examined μ MT^{-/-} mice that lack mature B cells. In μ MT^{-/-}, we did not detect any change in the numbers of BrdU-positive cells compared with μ MT^{+/-} littermates ($p = 0.358$; ANOVA, $p = 0.358$; $F_{2, 10} = 0.951$; Fig. 1A).

Depletion of CD4⁺ and CD3⁺, but not CD8⁺ T cells reduces adult hippocampal neurogenesis

We next investigated the role of CD4 vs CD8 T cells in maintaining baseline levels of adult neurogenesis. We used Abs to deplete

subsets of T cells in C57BL/6 wild-type animals. The animals received single injections of either anti-CD3, anti-CD4, anti-CD8, or anti-CD4/CD8 Abs compared with mice receiving injections of control IgG and 0.9% NaCl. Two days after subset-specific T cell depletion (Fig. 2A), mice received one injection of BrdU and were killed 24 h later.

Treatment with anti-CD3, anti-CD4, and anti-CD4/CD8 Abs led to significant decrease of the total numbers of BrdU-positive cells by 15–30% (vehicle vs anti-CD3, $p = 0.034$; vehicle vs anti-CD4, $p = 0.0005$; vehicle vs anti-CD4/CD8, $p = 0.0015$; ANOVA, $p < 0.0001$; $F_{4, 20} = 13.77$; Fig. 2B). Anti-IgG ($p = 0.2588$) or anti-CD8 treatment alone ($p = 0.2496$) did not change the level of cell proliferation. Anti-CD3 and anti-CD4 treatment led to a 40% decrease in BrdU-positive/DCX-positive immature neurons (vehicle vs anti-CD3, $p = 0.0076$; vehicle vs anti-CD4, $p = 0.0001$). Again, anti-CD8 treatment alone did not elicit a difference in the numbers of newborn DCX-positive neurons ($p = 0.5827$; ANOVA, $p < 0.0001$; $F_{4, 20} = 30.21$; ANOVA, $p = 0.015$; $F_{2, 10} = 9.351$; Fig. 2C).

To assess this effect on the survival of newly generated neurons in the DG, the treatment with anti-CD4 and anti-CD8 was repeated and the mice were killed 4 wk after BrdU injection. At this time point, numbers of BrdU-positive/NeuN-positive cells represent a measure of net neurogenesis. The anti-CD4 treatment led to a significant decrease in BrdU-positive cells (IgG-treated controls vs anti-CD4 treatment, $p = 0.0156$; ANOVA, $p = 0.029$; $F_{2, 10} = 4.954$) and a significant decrease in the number of BrdU-positive/NeuN-positive cells (IgG-treated controls vs anti-CD4 treatment, $p = 0.0156$).

To confirm our finding, we used CD4^{-/-} transgenic mice in addition to the Ab-depleted animals. We could confirm the decreasing effect of CD4 depletion on the number of BrdU-positive cells in the CD4^{-/-} mice (C57BL/6 vs anti-CD4, $p = 0.0005$; C57BL/6 vs CD4^{-/-} mice, 997 ± 145.22 , $p = 0.0001$; ANOVA, $p < 0.0001$; $F_{3, 15} = 47.78$; Fig. 2D).

CD4, but not CD8 repopulation increases adult hippocampal neurogenesis in RAG2^{-/-} mice

RAG2^{-/-} mice that lack functional T and B cells were repopulated with CD4 cells. This restoration normalized precursor cell proliferation, as measured by the total number of Ki67-positive cells (marking the cells in cell cycle) in the subgranular zone of the DG (RAG2^{-/-} NaCl vs RAG2^{-/-} CD4, 1998 ± 160 vs 2865 ± 298 , $n = 4$, $p = 0.0056$; Fig. 1B). In contrast, CD8 repopulation did not change the numbers of Ki67-positive cells (RAG2^{-/-} CD8, 1909 ± 313 ; $n = 4$). We obtained the same results using the neuronal progenitor marker DCX in Ki67-positive cells (RAG2^{-/-} NaCl vs RAG2^{-/-} CD4, 1288 ± 275 vs 1975 ± 319 , $n = 4$, $p = 0.017$; RAG2^{-/-} CD8, 1275 ± 206 ; Fig. 1C). These data further strengthen the result that CD4 T cells are the main T cell population responsible for the neurogenic effect.

The baseline difference between RAG2 and RAG1 KO animals is notable and may be based on the fact that RAG1, but not RAG2, is expressed in the brain. The investigation of the functional impact of the RAG1 gene in neurogenesis is interesting, but was not within the scope of our present study.

CD4-depleted mice show reduced BDNF production in the adult brain

To address one key candidate as mediator of the effects of peripheral CD4 T cells on the generation of newborn neurons in the adult hippocampus, we measured BDNF protein in brain tissue. In whole-brain extracts, BDNF production was significantly decreased in CD4-depleted animals (IgG-treated mice, 0.39 ± 0.02 pg/mg; anti-CD4-treated mice, 0.31 ± 0.004 pg/mg, $p = 0.0049$;

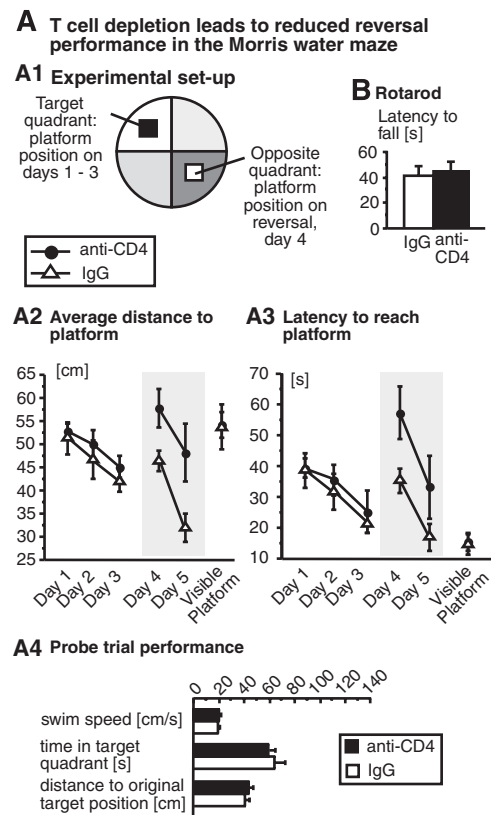


FIGURE 3. T cell-depleted mice show impaired performance in the MWM. *A*, Anti-CD4-treated mice vs IgG-treated controls (*A1*). During the acquisition phase between days 1 and 3, no significant differences between the groups were detected. Both groups learned the task (downward-sloping learning curve and a quadrant preference in the probe trial on day 4; *A2* and *A3*). CD4-depleted mice showed a significantly reduced performance during the reversal training (*A2* and *A3*). Both groups again performed at the same level, when the platform was made visible on day 6 (*A4*). *B*, No differences between CD4 depleted and controls in the rotarod test.

ANOVA, $p = 0.0049$; $F_{2, 10} = 26.74$). In contrast, no difference in NGF production was found (IgG-treated mice, 1.94 ± 0.13 pg/mg; anti-CD4-treated mice, 1.84 ± 0.09 pg/mg, $p = 0.5365$; ANOVA, $p = 0.537$; $F_{2, 10} = 0.417$; Fig. 2G).

CD4 T cell depletion leads to decreased hippocampal neurogenesis, but the numbers of newborn cells can be increased by voluntary physical activity

To date, our data indicated that the depletion or genetic ablation of CD4-positive T cells leads to a significant decrease in proliferating neuronal precursor cells and net neurogenesis. We next studied whether voluntary wheel running would still lead to an increase of proliferating precursor cells in CD4-depleted/deficient animals, as it is known from naive mice (14).

In this study, we used both CD4^{-/-} transgenic mice and mice treated with anti-CD4 Ab. Despite their low baseline levels of cell proliferation in the DG, both types of CD4-depleted/deficient mice responded to physical activity (control vs anti-CD4, $p = 0.0106$; control vs CD4^{-/-}, $p = 0.0001$; ANOVA, $p < 0.0002$; $F_{3, 15} = 26.47$; Fig. 2E). Thus, the lack of CD4-positive T cell function led to a decreased baseline of cell proliferation, but maintained activity-dependent regulation. When mice that lack the common γ -chain on the RAG2^{-/-} background and thus are devoid of functional T, B, and NK cells were allowed to run on a running wheel, the normal neurogenic effect of physical activity was absent (control, 1308 ± 172.65 vs common γ ^{-/-}, 1757.6 ± 341.36 , $p = 0.2953$).

Behavioral consequences of CD4 T cell depletion

We have previously found a positive association between the baseline level of adult neurogenesis and parameters that describe the acquisition of the water maze (MWM) task (33).

We compared anti-CD4-treated mice with IgG-treated controls in the MWM. During the acquisition phase between days 1 and 3, no significant differences between CD4-depleted mice and controls were detected, which was reflected by the downward-sloping learning curve and a quadrant preference in the probe trial on day 4 ($p = 0.01$; Fig. 3A). However, in the CD4-depleted mice, a significantly impaired performance was detected during the reversal learning (with the hidden platform at a new position), with regard to both latency (anti-CD4, 36.13 ± 10.94 ; control, 16.88 ± 4.21 , $p = 0.002$; ANOVA, $F_{2,16}$ 4.72) and distance to platform (anti-CD4, 49.4 ± 6.76 ; control, 31.93 ± 2.94 ; $p = 0.002$; ANOVA; $F_{2,16}$ 4.80; Fig. 3A). Both groups again performed at the same level when the platform was made visible on day 6 (Fig. 3A). A rotarod test to assess general locomotor functions and fitness was performed on day 7. Both groups performed on the same level on the rotarod (IgG control group, 60.47 ± 5.844 s; CD4 group, 66.82 ± 6.17 s; $p = 0.46$; ANOVA; $F_{2,16}$ 0.56; Fig. 3B).

Discussion

Our findings support the key role of an intact immune system in the maintenance of a neurogenic homeostasis in the hippocampus and indicate that combined T and B cell loss leads to decreased cell proliferation in the adult DG, whereas no such effect was seen for selective B cell deficiency. This is in line with the reports of hippocampus-related behavioral deficits, including performance in the MWM in SCID and nude mice, both lacking functional T and B cells and showing reduced adult hippocampal neurogenesis (22). Because the lack of B cells only had no effect on neurogenesis levels, our data point toward the T cells being the key players.

Moreover, we showed that only the absence of CD4, but not CD8 T cells in the periphery decreased cell proliferation and neurogenesis in the DG. Along the same line, repopulation of RAG2^{-/-} mice with CD4, but not CD8 cells brought the number of proliferating precursor cells (Ki67 and DCX positive) back toward a level comparable to C57BL/6 wild-type mice. Thus, the present data implicate a key role of CD4-positive T lymphocytes in the maintenance and control of adult hippocampal neurogenesis under physiological brain conditions. This differential effect was consistent with reports that only CD4-positive T cells seem to have a neuroprotective effect on facial motor-neuron survival after peripheral nerve injury (34, 35).

We found decreased BDNF production in the brains of the CD4-depleted mice, whereas NGF production did not change. BDNF has been linked to the survival and functional differentiation of multiple neuronal populations (36), but no obvious effect of NGF on adult neurogenesis *in vivo* had been found previously (37). NGF served us in this study as an internal control of general non-specific increase of neurotrophic factors. These data suggest that the decrease in adult neurogenesis in the DG of CD4-depleted mice could be partially caused by decreased BDNF production in the brain. It has been proposed that CNS-specific T cells were the source of additional BDNF, and thereby might enhance adult neurogenesis (38). Under physiological conditions only few T cells are found in the brain, although T cell trafficking to the brain does occur constantly. An even smaller proportion of MPB-specific T cells leaves the thymus and circulates in the periphery (21). It seems unlikely that under physiological conditions these minute numbers of T cells should represent a significant source of regulatory BDNF within the brain. In this study, we show that CD4-

positive T cells, regardless of their Ag specificity, are involved in maintaining physiological BDNF levels in the brain and contribute to the neurogenic microenvironment without actually infiltrating the healthy brain. Despite the fact that T cells might release BDNF, the main source of BDNF that is released in response to T cell activation will come from other cellular sources and involve a bystander effect from neural cells (24, 25).

The identification of BDNF as potential mediator of CD4-dependent baseline regulation of adult neurogenesis brings up many questions, as follows. Which cell populations are the source of BDNF? How is the BDNF release mediated? What is the kinetics of this effect? And how does this pathway relate to other possible mediators? We used NGF as internal control and did not find any effect of NGF, but other soluble factors, including cytokines might be involved in addition to BDNF. This also applies to the role of CD4-dependent BDNF release in the context of the neurogenic effects of physical activity. The answers to these questions were beyond the scope of the present study, but need to be addressed in future experiments.

The decrease in precursor cell proliferation became apparent within only 24 h after lymphocyte depletion, suggesting the disturbance of an otherwise highly balanced system maintaining neurogenesis at physiological levels. In contrast to other examples of regulated neurogenesis that occur quickly after the stimulus, e.g., up-regulation after voluntary wheel running (39) or down-regulation after stress (40), in this study we find the consequences of discontinuing immunological support for baseline neurogenesis.

Despite low baseline levels of neurogenesis in the CD4-depleted and CD4 KO mice, voluntary wheel running, a strong stimulus for adult neurogenesis in wild-type mice (14), could still enhance neurogenesis. When we used a common γ -chain, KO animals on a RAG2^{-/-} background that lack functional T, B, and NK cells, however, this neurogenic effect of exercise was lost. Thus, it seems possible that CD4 T cells are the main component of the adaptive immune system to control baseline neurogenesis, whereas the concert of the entire pool of adaptive immune cells is needed to mount a response to neurogenic stimuli.

To assess the functional impact of CD4 depletion on learning and memory, the mice were tested in the MWM. CD4-depleted mice showed a normal learning curve, but had deficits in the reversal learning task. It has been proposed that reversal learning involves reconsolidation and relearning rather than the extinction of the original memory (41, 42). Our present data suggest that CD4 depletion causes deficits in such reversal situation only. This would be in line with our hypothesis that new neurons are not needed for learning and memory *per se*, but to avoid catastrophic interference in relearning situations (13). SCID and nude mice that lack both B and T cells showed impairments during both acquisition and reversal of the water maze task (22). Our findings thus provide an explicit hint for a distinct effect of CD4-positive T cells during memory reconsolidation and relearning.

Taken together, our results imply that an intact immune system is needed to maintain adult neurogenesis at a physiological level. This is in line with results by others using SCID and nude mice (22). In the current study, we took these findings a step further, and identified CD4-positive T cells as the link between the adaptive immune system and adult hippocampal neurogenesis on a cellular and functional level. Moreover, we could show that the presence of NK cells relates to the neurogenic effect of physical activity. Not only pathologies that include neuroinflammation like Alzheimer and multiple sclerosis show impaired neurogenesis that has been linked to the decline in cognitive function (43–45). Also, in neuropsychiatric diseases without a neuroinflammatory phenotype,

such as schizophrenia and major depression, decreased neurogenesis has been shown (46). Interestingly, the immune function, mainly IL-2 production, is also impaired in these psychological disorders (47), showing that a close link between immune and cognitive functions is not too far-fetched. Sparing CD4 T cell and NK cell function during a chronic immune-suppressive treatment might help to avoid side effects on adult neurogenesis, and thus, possibly on cognition and mental health. Conversely, targeting CD4 cells might provide a therapeutic tool to treat neurological and psychological dysfunctions.

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Disclosures

The authors have no financial conflict of interest.

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