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Age-Dependent Spatial Memory Loss Can Be Partially Restored by Immune Activation

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Abstract

Aging is often associated with a decline in hippocampus-dependent spatial memory. Here, we show that functional cell-mediated immunity is required for the maintenance of hippocampus-dependent spatial memory. Sudden imposition of immune compromise in young mice caused spatial memory impairment, whereas immune reconstitution reversed memory deficit in immune-deficient mice. Analysis of hippocampal gene expression suggested that immune-dependent spatial memory performance was associated with the expression of insulin-like growth factor (*Igf1*) and of genes encoding proteins related to presynaptic activity (*Syt10*, *Cplx2*). We further showed that memory loss in aged mice could be attributed to age-related attenuation of the immune response and could be reversed by immune system activation. Homeostatic-driven proliferation of lymphocytes, which expands the existing T cell repertoire, restored spatial memory deficits in aged mice. Thus, our results identify a novel function of the immune system in the maintenance of spatial memory and suggest an original approach for arresting or reversing age-associated memory loss.

Introduction

With increased life expectancy, there has been a profound growth in the segment of the population that suffers from age-related impaired cognitive ability. Thus, massive research efforts are underway to develop approaches to prevent or ameliorate brain aging, and successful therapies are expected to have significant impact on the quality of life of affected individuals. Of the brain regions affected by aging, the hippocampus is particularly vulnerable,[1] and age-related decreases in hippocampus-dependent spatial and episodic memory capacity have been described.[2]

Recently, we have shown that peripheral immune cells have a pivotal role in maintaining hippocampal plasticity in healthy adult central nervous system (CNS).[3] Taken together with the decrease in cellular immunity that occurs with progressive aging,[4] these findings provided the basis for the present study of the relationships between age-related hippocampal-dependent memory loss and aging of the immune system. Specifically, we wished to determine whether immune compromise contributes to hippocampal-dependent spatial memory deficits, and, if so, whether this effect is reversible. Moreover, we wished to determine if aging of the immune system is a factor in age-related memory loss and whether restoration of the immune system would enhance spatial memory.

Materials and Methods

Animals

Inbred male C57BL/6 wild type and prkdc severe combined immunodeficient (SCID) mice were supplied by the Animal Breeding Center of the Weizmann Institute of Science (young mice) or by the National Institute of Aging (NIA; Bethesda, MD) (aged mice). Aged mice were allowed a 3-month adaptation period following shipment from the NIA to our laboratory. The cages were placed in a light- and temperature-controlled room, and all behavioral tests were conducted during the dark hours. All animals were handled according to the regulations formulated by the Weizmann Institute's Animal Care and Use Committee, and maintained in a pathogen free environment.

Morris water maze behavioral test

For the acquisition phase, mice were given three to four trials per day on 5 consecutive days. In each trial, the mice were

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required to find a hidden platform located 1.5 cm below the water surface in a 1.1-m-diameter pool (Morris water maze). Within the testing room, only distal visual–spatial cues for location of the submerged platform were available. The escape latency, i.e., the time required by the mouse to find the platform and climb onto it, was recorded for up to 60 sec. Each mouse was allowed to remain on the platform for 20 sec and was then moved from the maze. If the mouse did not find the platform within 60 sec, it was manually placed on the platform for 20 sec. The interval between trials was 10 min. In the probe trial phase, the platform was removed from the pool and the location of each mouse in the pool was followed for 60 sec. Data were recorded using an EthoVision automated tracking system (Noldus).

Location novelty and object recognition

Mice were individually habituated to a 40 × 40-cm square-shaped open field with 30-cm-high Plexiglas walls (made opaque at the bottom 20 cm from the base), for 10 min for 6 consecutive days. On the days 7 and 8, the mice were familiarized with two identical plastic objects that were placed in the open field, one in each corner, 8.5 cm from the walls. Each session lasted 5 min. On day 8, 30 min after familiarization, mice were placed again in the open field, and their exploratory behavior was measured. Each session lasted at least 10 min, and up to 20 min, until a total exploration time of 20 sec was achieved. On day 9, one of the objects was moved to another corner in the arena, and the exploratory behavior of each mouse was tested for 10 min.

Following the novel-place test trial, the mice were returned to their cages for 20–30 min and then placed in the arena for a novel object recognition test, in which one of the objects was replaced with a novel object that differed in shape, color, and texture. All objects and the arena were thoroughly cleaned with 10% ethanol between trials to remove odors. The time spent exploring each object during the familiarization training and the testing trials was recorded by a trained observer who was blinded to the genotype and treatment of the animal. 'Exploration' was defined as approaching the object nose-first within 3 cm, sniffing, and touching the object with the tip of the nose and/or with the paws. Standing next to the object or on top of it was not considered as explorative activity. The time spent exploring each object, as a percentage of the total exploration time, was calculated for each trial. The difference between the percentage of time spent exploring the object at its novel location (day 9) and the percentage of time spent exploring the same object at its original location on the previous day (day 8) was calculated as a measure of location novelty recognition. Results were presented as the preference (percent of time) to explore the object with a novel location, beyond the time spent (percent) exploring the same object in its prior location. The time spent exploring a novel object was defined as the absolute preference to explore the novel object during the test trial (on day 9). Velocity and total distance moved during the test were measured using an EthoVision automated tracking system (Noldus).

Irradiation and bone-marrow transplantation

C57BL/6 wild-type and SCID mice underwent total body γ-irradiation from a cobalt source according to the following protocols: for SCID mice, one dose of 300 rad; for young (8-week-old) wild-type mice, 450 rad, followed by 950 rad on the next day; for old (15-month-old) wild-type mice, one dose of 950 rad. In all the experiments, the heads were shielded during irradiation to avoid radiation-induced brain damage. No infiltration of bone marrow-derived cells into the brain was seen in chimeric mice that were created with head shielding during the process of irradiation (not shown). Bone marrow transplantation was performed 1 day after the last irradiation, by intravenous injection of 4–5 $\times 10^6$ bone-marrow cells suspended in phosphate-buffered saline (PBS) (total volume 0.15 mL).

Immunofluorescence staining for flow cytometry

Freshly isolated spleen cells were washed with fluorescence-activated cell sorting (FACS) buffer (PBS containing 2% fetal calf serum) and incubated with anti-CD45.1 Allophycocyanin (APC) and anti-CD45.2 Phycoerythrin (PE) (eBioscience), for 30 min at 4°C. Samples were then washed twice with staining buffer and subjected to FACS analysis.

RNA purification, cDNA synthesis, reverse transcription PCR, and real-time quantitative PCR

Cells from whole hippocampus or spleen were extracted with TRI reagent (MRC, Cincinnati, OH), and total cellular mRNA was purified from the lysates using the RNeasy kit (Qiagen, Hilden, Germany). mRNA (1 μg) was converted to cDNA using SuperScript II (Promega, Madison, WI). The expression of specific mRNAs was assayed using fluorescence-based real-time quantitative PCR (Q-PCR) with selected gene-specific primer pairs. Q-PCR reactions were performed using Absolute™ Q-PCR SYBR® Green ROX mix (ABgene). Q-PCR products were detected by SYBR Green I (Roche Molecular Biochemicals, Indianapolis, IN), obtained in triplicate for each of the cDNA samples using the Rotor-Gene 6 instrument (Corbett Research), and analyzed using Rotor-Gene 6000 software (version 1.7, Corbett). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was chosen as a reference gene. The amplification cycle was 95°C for 5 sec, 60°C for 20 sec, and 72°C for 15 sec. At the end of the assay, a melting curve was constructed to evaluate the specificity of the reaction. The following primers were used:

Gene	*Forward 5′-3′*	*Reverse 5′-3′*
gapdh	AATGTGTCCGTCGTGGATCTGA	GATGCCTGCTTCACCACCTTCT
Syt10	TGAGGGCCGAAGACTTAAAA	GCTTCGTTGTACACGGGATT
Cplx2	AAGAGCGCAAGGCGAAACA	TGGCAGATATTTGAGCACTGTG
Igf1	CCGGACCAGAGACCCTTTG	CCTGTGGGCTTGTTGAAGTAAAA
Ifn-γ	ACAGCAAGGCGAAAAAGGA	TGGACCACTCGGATGAGC

Statistical analysis

The STATISTICA statistics package was used to perform statistical analysis of all data. Data that included three groups were analyzed using either one-way or two-way analysis of variance (ANOVA) with group treatment and number of days as the between subject factors. Significant main effects were further evaluated using Fisher least significant difference (LSD) or Tukey–Kramer honestly significant difference (HSD) *post hoc* analysis. Data from experiments that included only two groups were analyzed using a two-tailed Student *t*-test, with group treatment or immune background as the between-subject factor.

Results

Impaired hippocampus-dependent spatial memory in immune-deficient mice and its reversal by bone marrow transplantation

Impaired T cell immunity is associated with impaired performance in the hippocampus-dependent spatial learning/memory task in the Morris water maze.[3,5–7]. Here, we wished to test whether T cells contribute to the processes involved in hippocampus-dependent spatial memory abilities, learning skills, or both. We used wild-type and immune-deficient mice (SCID) on the C57BL/6 background. In the ac-

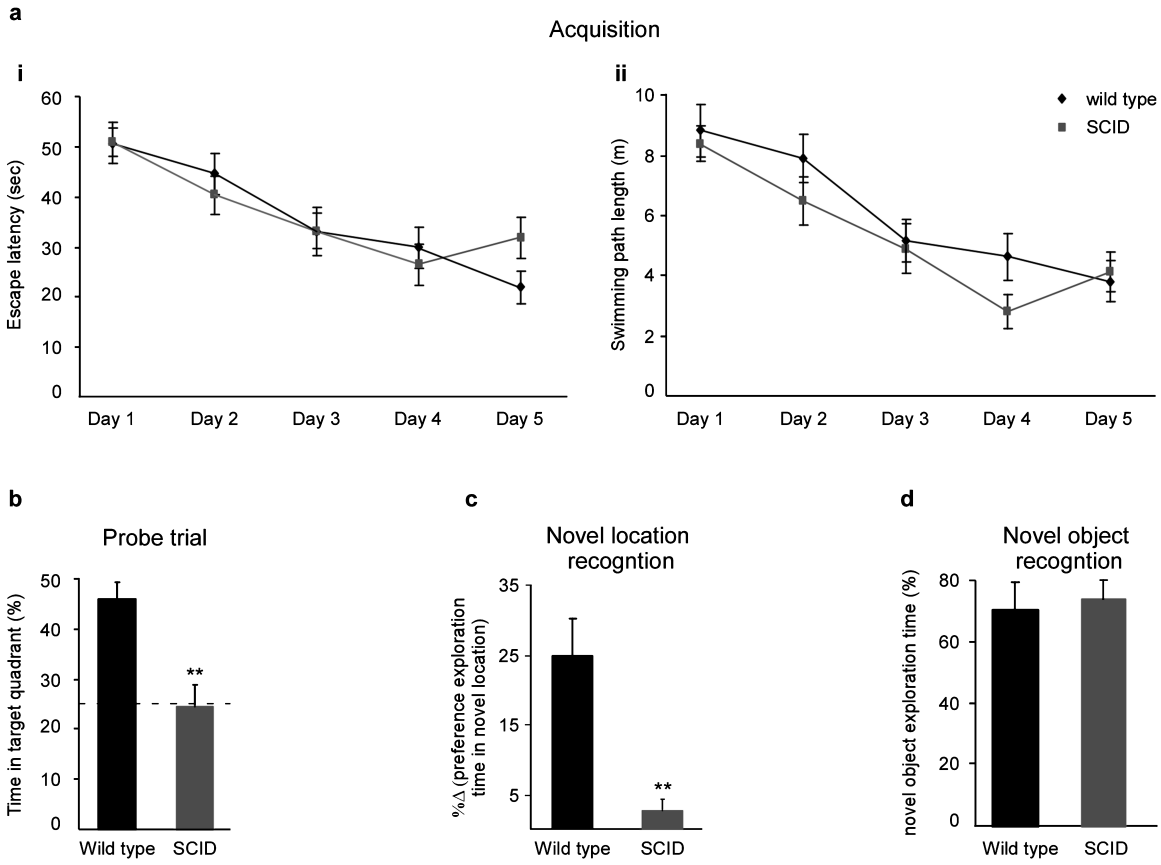

FIG. 1. Spatial memory is impaired in immune-deficient mice. (**a** and **b**) C57BL/6 (SCID and wild-type) mice were tested in the Morris water maze spatial learning/memory task. (**a)** Average escape latency (**i**) and path length to platform (**ii**) during acquisition. Each point represents the average of four trials a day. No differences were found between the groups (two-way-repeated-measures ANOVA; escape latency, groups, $F_{1,7} = 0.13$, $p = 0.91$; days, $F_{4,28} = 15.84$, $p = 0.0001$; groups X days, $F_{4,28} = 1.1$, $p = 0.376$; path length, groups, $F_{2,19} = 0.88$, $p = 0.43$; days: $F_{4,76} = 29.63$, $p < 10^{-7}$; groups × days, $F_{8,76} = 1.64$, $p = 0.13$). (**b**) A probe trial was conducted 4 days after acquisition. Bars represent performance in the probe trial, calculated as percentage of time spent in the target quadrant, which formerly contained the platform, of the total 60 sec spent in the maze ($t_7 = 3.61$, $^{**}p = 0.0086$; $n = 5$ and $n = 4$ for wild-type and SCID mice, respectively). Note that, on the basis of a random choice, mice are expected to spend 25% of the time in each quadrant (dashed line). Importantly, the groups did not differ in their motor abilities, indicated by swimming velocity during the probe trial ($t_7 = -0.97$, $p = 0.36$). (**c**) C57BL/6 SCID and wild-type mice were tested in the novel location recognition task. The preference exploration time of the object in its novel location is presented as the percentage of time spent exploring the object in the new location beyond the time spent in the old location. SCID mice showed a significantly lower preference relative to wild-type ($t_{5.9} = -3.8$, $^{**}p = 0.008$, $n = 8$ and $n = 6$ for SCID and wild-type mice, respectively). No difference was found in total exploration time among the groups. (**d**) C57BL/6 SCID and wild-type mice were tested in the novel object recognition task. The preference exploration time of the novel object was calculated out of the total exploration time in the test trial. No differences were found between the groups ($t_{13} = 0.85$, $p = 0.41$, $n = 8$ and $n = 7$ for SCID and wild-type mice, respectively). Error bars represent standard error of the mean (SEM).

quisition stage (which measures the ability to learn the location of a hidden platform), the SCID mice showed similar behavior to that of the wild-type mice (Fig. 1a). However, in the probe trial (which measures the ability to remember the location of the platform), which was conducted 4 days after acquisition was terminated, a significant impairment in spatial memory was observed in the SCID mice, manifested by the reduced time spent searching for the platform in its original location (Fig. 1b). To substantiate our results further, we assessed the ability of SCID mice to recognize a novel spatial arrangement of familiar objects, a task that measures spatial memory and is considered to be hippocampus-dependent.[8] SCID mice showed a significant reduction in their preference for exploring the object in a novel location, compared to wild-type control mice (Fig. 1c). In contrast, no difference was found between wild-type and SCID mice in a short-delay version of the object recognition task, known to be unaffected by hippocampal damage in mice[9] (Fig. 1d).

To provide direct functional evidence that the impaired memory exhibited by the immune-deficient mice (Fig. 1a–c) could be attributed to the immunological deficit, we tested whether overcoming the immunological deficit could reverse the memory impairment. For this purpose, we used C57BL/6 SCID mice in which we replaced the bone marrow with that of wild-type mice. To create such chimera, C57BL/6 SCID ($CD45.2^+$) mice underwent mild total body irradiation (while their heads were protected by a lead shield to avoid radiation-induced damage[10]), and were transplanted with bone marrow cells harvested from B6/SJL wild-type mice ($CD45.1^+$). CD45 polymorphism was used to assess the degree of chimerism. As controls, we used SCID mice transplanted with bone marrow cells isolated from identical SCID mice, as well as wild-type mice.

After 8 weeks, the mice were trained and tested in the Morris water maze for their spatial learning and memory abilities. No differences in learning abilities were found among SCID mice transplanted with SCID-bone marrow ($CD45.2^+$), SCID mice transplanted with B6/SJL wild type-bone marrow ($CD45.1^+$) and nontreated wild-type mice (acquisition; Fig. 2a). However, when the same animals were tested in the probe trial 3 days after acquisition was terminated, the SCID mice that received wild-type bone marrow cells showed normal spatial memory abilities (Fig. 2b), whereas the SCID mice transplanted with SCID-bone marrow, which served as a control for the transplantation procedure, showed spatial memory impairment (Fig. 2b), similar to nontransplanted SCID mice (Fig.1b). Figure 2c verifies the success of the immune reconstitution by showing that the vast majority of immune cells found in SCID (CD45.2) mice transplanted with wild type-bone marrow (CD45.1) originated from the donor bone marrow (>95% chimerism). As an additional parameter to measure the success of the immune reconstitution, the spleen size of all animals was examined, following completion of cognitive testing; the group with the worst memory performance also possessed the smallest average spleen size (Fig. 2d).

Next, we tested the mice of the three groups for their hippocampal expression of *Syt10* and *Cplx2*, two genes encoding presynaptic proteins that are involved in fast neurotransmitter release and may contribute to memory performance.[11] The choice of these genes was based on screening of hippocampal mRNA for genes whose expression was altered in T cell-deficient mice (Supplementary Table 1). To determine if the immune reconstitution described above affected expression of *Syt10* and *Cplx2*, hippocampus samples were collected 24 hr following the memory test (i.e., the probe trial) and analyzed for gene expression by Q-PCR. Immune-deficient mice with impaired memory expressed lower levels of both *Syt10* and *Cplx2*, relative to normal animals with normal memory skills (Fig. 2e,f). However, in the hippocampi of the SCID mice transplanted with wild-type bone marrow, the levels of these genes were restored to normal, correlating with restoration of memory (Fig. 2e,f).

Finally, we tested the same samples for expression of *Igf1*, a gene whose expression was found to be both immune and experience dependent (Supplementary Table 1), and has a well established role in hippocampal-dependent spatial memory performance.[12] Q-PCR showed downregulation of hippocampal *Igf1* expression in the immune-deficient mice compared to wild type, and full restoration in the SCID mice transplanted with wild-type bone marrow (Fig. 2g). Together, these results demonstrate that an immune-related memory deficit is reversible upon immune restoration.

Imposition of immune deficiency at adulthood is sufficient to cause spatial memory loss

The memory deficit found in the immune-compromised mice prompted us to consider that immune deterioration might be a factor in age-related memory loss. To address this issue, it was essential to determine whether a reduction in immunity in adulthood would be sufficient to cause a deficit in spatial memory abilities. Immune compromise was imposed on wild-type mice in adulthood, by replacing their normal immune cell repertoire with immune cells derived from age-matched immune-deficient mice. Young (8-week-old) C57BL/6 wild-type mice were lethally irradiated (with head shielding) and transplanted with bone marrow cells taken from SCID mice on the same genetic background. As controls, we used naïve wild-type mice as well as irradiated wild-type mice of the same age that were transplanted with bone marrow cells derived from syngeneic wild-type mice. Behavioral experiments were performed 8 weeks after the bone marrow transplantation. In the Morris water maze task, learning was not affected by the manipulations of peripheral immunity, as no differences in acquisition were found between naive wild-type mice, wild-type mice transplanted with wild-type bone marrow and wild-type mice transplanted with SCID bone marrow (Fig. 3a). However, when the mice in which immune deficiency was imposed (transplanted with SCID bone marrow) were tested in the probe trial, they showed almost no memory for the platform location, unlike the group transplanted with wild-type bone marrow ($28 \pm 2\%$ of the time spent in the target quadrant vs. $38 \pm 4\%$; $p < 0.05$) (Fig. 3b). To substantiate our results further, we also tested the mice using the novel location task. Similar to SCID mice (Fig. 1), the wild-type mice in which immune deficiency was imposed showed a profound reduction in their preference for exploring an object in its novel location, compared to wild-type mice transplanted with normal bone marrow (Fig. 3c). When tested in the short-delay version of the novel object recognition task, both groups demonstrated preference for exploration of the novel object

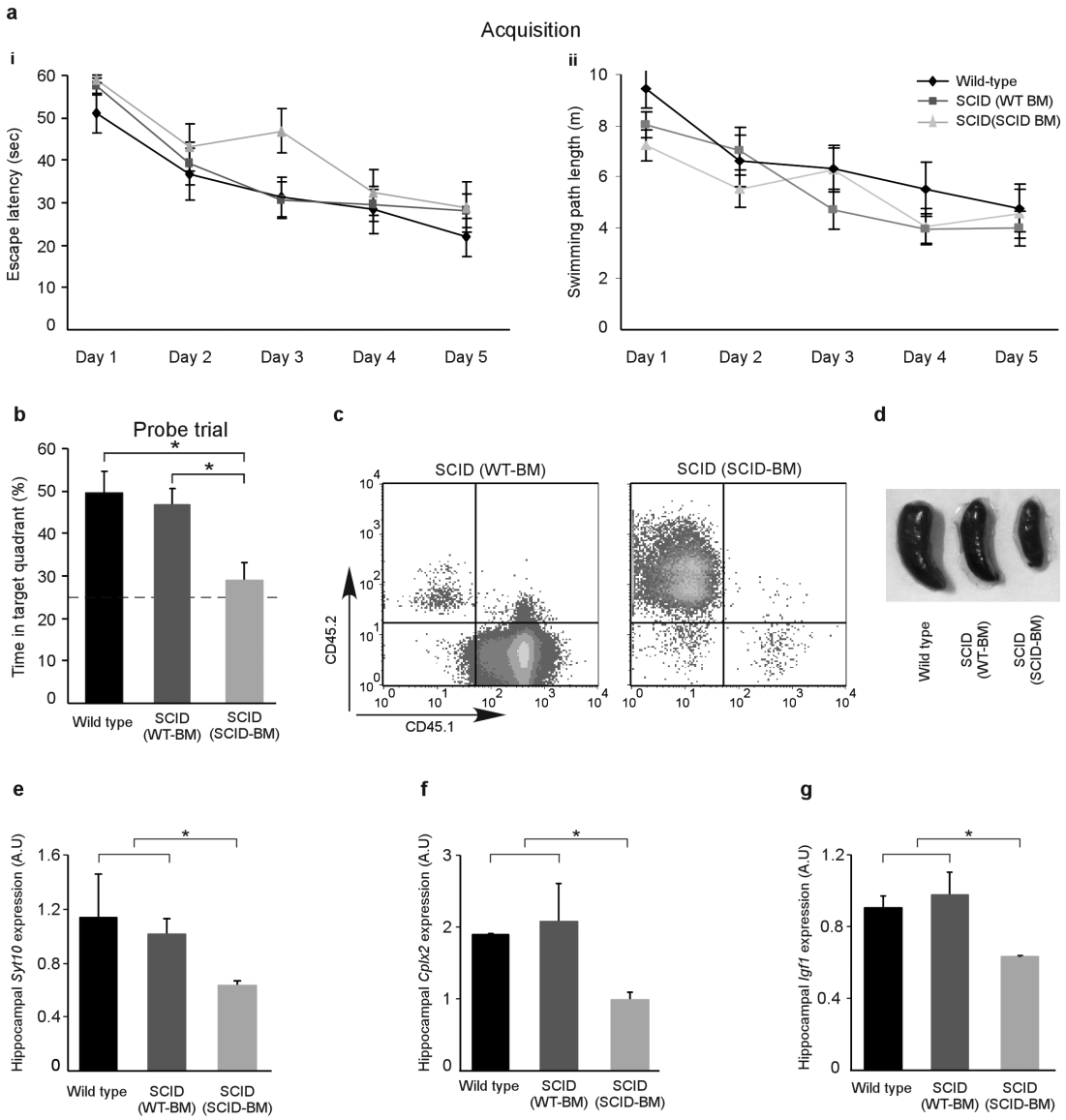

FIG. 2. Spatial memory impairment in immune deficient mice is restored upon immune reconstitution. (**a–g**) C57BL/6 SCID mice were transplanted with bone marrow cells derived from age-matched C57BL/6 wild type mice SCID[WT BM] or with bone marrow cells derived from syngeneic SCID mice SCID[SCID BM]. The transplanted mice, as well as wild-type untreated controls, were tested in the learning/memory task in the Morris water maze. (**a**) Average escape latency (**i**) and path length to platform (**ii**) during acquisition. Each point represents the average of three trials a day. No differences were found between the groups. (Two-way-repeated-measures ANOVA; escape latency, groups, $F_{2,15} = 1.38$, $p = 0.28$; days, $F_{4,60} = 16.12$, $p = 0.0001$; groups × days: $F_{8,60} = 0.45$, $P = 0.88$; path length, groups, $F_{2,15} = 2.55$, $p = 0.11$; days, $F_{4,60} = 12.58$, $p = 0.0001$; groups × days: $F_{8,60} = 0.62$, $p = 0.76$). **(b)** A probe test was performed 3 days after the last acquisition day. Bars represent performance in the probe trial, calculated as in (Fig. 1b). A one-way ANOVA ($F_{2,15} = 5.96$, $p = 0.01$; $^*p < 0.05$; Tukey–Kramer HSD *post hoc* analysis; $n = 6$ for wild-type controls and $n = 5$, or 7 for SCID[SCID-BM] and SCID[WT-BM], respectively). Analysis of the swimming velocity during probe trial showed a significant difference only between the wild-type controls and the SCID[WT-BM]. (A one-way ANOVA indicated a significant difference between the groups [$F_{2,15} = 5.88$, $p = 0.01$; $^*p < 0.05$; Tukey–Kramer HSD *post hoc* analysis]**). (c)** FACS analysis of CD45 polymorphism demonstrating the successful chimerism obtained by transplantation of BM cells derived from B6/SJL wild-type mice ($CD45.1^+$) into C57BL/6 SCID mice ($CD45.2^+$). **(d)** Representative photographs of spleens removed from mice of the three groups show that spleen sizes correlated with immune reconstitution and memory abilities shown in *b*. **(e–g)** Immune reconstitution restored hippocampal expression of the genes encoding presynaptic proteins: *Syt10* and *Cplx2*, and of *Igf1* in SCID mice. Hippocampal expression levels of: (**e**) *Syt10* ($t_{4.55} = -3.49$; $^*p = 0.020$), (**f**) *Cplx2* ($t_6 = -2.56$; $^*p = 0.042$), and (**g**) *Igf1* ($t_6 = -2.56$; $^*p = 0.045$), 24 hr following memory test. Expression was assessed by quantitative real-time PCR. Values represent the specified expression ratios in arbitrary units (A.U.), normalized against GAPDH in the same samples. ($n = 3$ animals per group; each was tested in triplicate.) Error bars represent SEM.

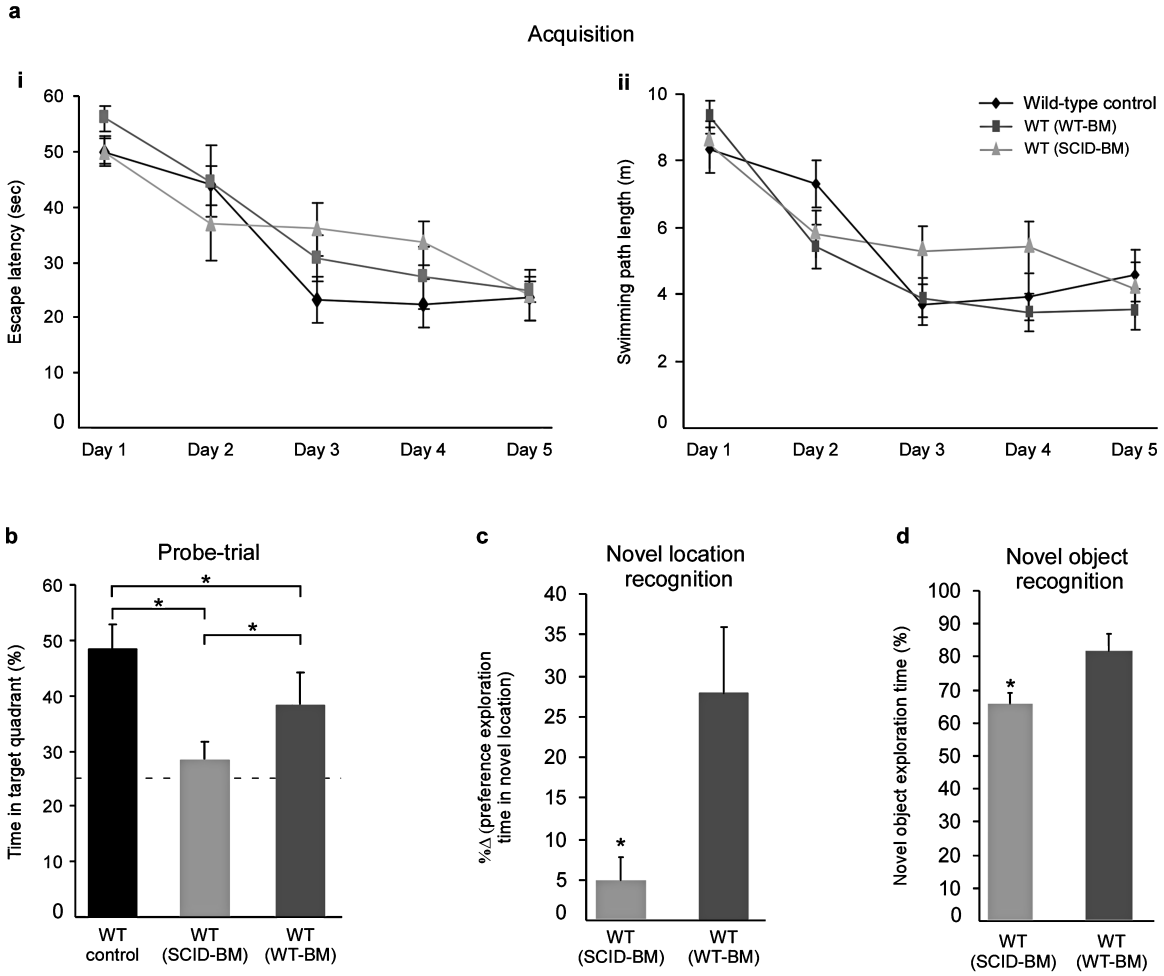

FIG. 3. Spatial memory is impaired in wild-type mice in which T cell immunity was compromised during adulthood. C57BL/6 wild-type mice were transplanted with bone marrow cells derived from C57BL/6 SCID WT[SCID-BM] or with bone marrow cells from syngeneic wild-type mice WT[WT-BM]. The transplanted mice as well as wild-type nontreated controls were tested in the learning/memory task in the Morris water maze (one of two independent experiments is shown). (**a**) Average escape latency (**i**) and path length to platform (**ii**) during acquisition. Each point represents the average of four trials a day. No differences were found between the groups (two-way-repeated-measures ANOVA; escape latency, groups, $F_{2,19} = 1.25$, $p = 0.3$; days, $F_{4,76} = 4.36$, $p = 0.0001$; groups × days, $F_{8,76} = 1.1$, $p = 0.36$; path length, groups, $F_{1,9} = 1.42$, $p = 0.26$; days, $F_{4,36} = 22.58$, $p < 10^{-7}$; groups × days: $F_{4,36} = 1.01$, $p = 0.38$). (**b**) Bars represent performance in the probe trial 1 day after acquisition (according to preliminary data we had showing differences in probe trial performance among groups identical to the above as soon as 1 day after acquisition), calculated as in Fig. 1b. A one-way ANOVA indicated a significant difference between treatment groups ($F_{2,19} = 12.41$, $p = 0.00036$; $^*p < 0.05$; Fisher LSD *post hoc* analysis; $n = 8$ for wild-type controls and $n = 7$ for wild type-BM and SCID-BM. Importantly, there were no differences in motor abilities among the groups, as indicated by swimming velocity during the probe trial (mean values ± SD: wild-type controls − 18.6 ± 2.4 cm/sec; wild-type-BM − 17.5 ± 1.6 cm/sec; and SCID-BM − 19.8 ± 2.4 cm/sec; $F_{2,19} = 2.01$, $p = 0.16$, one-way ANOVA). (**c**) WT(SCID-BM) mice showed impaired ability to recognize a novel location. Novel location exploration was calculated as in Fig. 1c ($t_{6.1} = -2.7$, $^*p = 0.03$; $n = 6$ and $n = 8$ for wild-type bone marrow and SCID bone marrow mice, respectively). No difference was found in total exploration time among the groups. (**d**) A smaller difference was found between WT(SCID-BM) and WT[WT-BM] mice in novel object recognition ($t_{13} = 2.60$, $^*p = 0.022$; $n = 7$ and $n = 8$ for wild-type bone marrow and SCID bone marrow mice, respectively). Exploration preference was calculated as in Fig. 1d. Error bars represent SEM.

(>50% of the time). However, the mice with the imposed immune deficiency showed reduced preference (Fig. 3d). T cell proliferation and FACS analyses revealed that wild-type animals in which a sudden immune compromise was imposed at adulthood, expressed features reminiscent of aged T cell immunity: a normal sized T cell population with a reduced capacity to proliferate[13] (Supplementary Table 2).

Immune activation reversed memory loss in elderly

By demonstrating that imposition of immune deficiency caused a memory deficit in young animals, our results suggest the possibility that immune senescence could be a causative factor of age-related memory loss. If this is indeed the case, the reduced ability of T cells in the elderly to sup-

port spatial memory might be due to the accumulation of suppressor T cells.[14] Accordingly, a reduction in suppressor-cell activity might constitute an approach to revive the capacity of aged lymphocytes to support spatial memory. To test this possibility, we studied the ability of peripheral lymphocytes from aged animals to restore an immune-related memory deficit under conditions that reduce the influence of suppressor cells. To this end, we transferred lymphocytes derived from old mice to SCID mice, which suffer from a memory deficit (Fig. 1). The use of SCID mice provided a lymphopenic environment that induces homeostatic-driven proliferation of the transferred lymphocytes, in part by overcoming the effect of suppressor T cells that exist in the aged animals.[15,16] In this experiment, we used the same mice that previously exhibited a memory deficit in novel location exploration task, as well as the same wild-type controls (Fig. 1c). The SCID mice replenished with lymphocytes derived from aged animals as well as their wild-type controls were tested in the Morris water maze 4 weeks following the lymphocyte transfer and showed a similar acquisition curve (Fig. 4a). However, in contrast to their previous performance in a spatial memory test (Fig. 1c), when tested in the probe trial following lymphocyte transfer, the spatial memory ability of the reconstituted SCID mice was identical to that of wild-type mice (Fig. 4b). To verify that suppressor cell activity was abrogated, and that lymphocyte activation took place as a result of the aged-lymphocyte transfer into the SCID mice,[17] we measured mRNA levels of interferon-γ (IFN-γ, a major T cell-derived cytokine.

Analysis of the spleens that were taken from the mice fol-

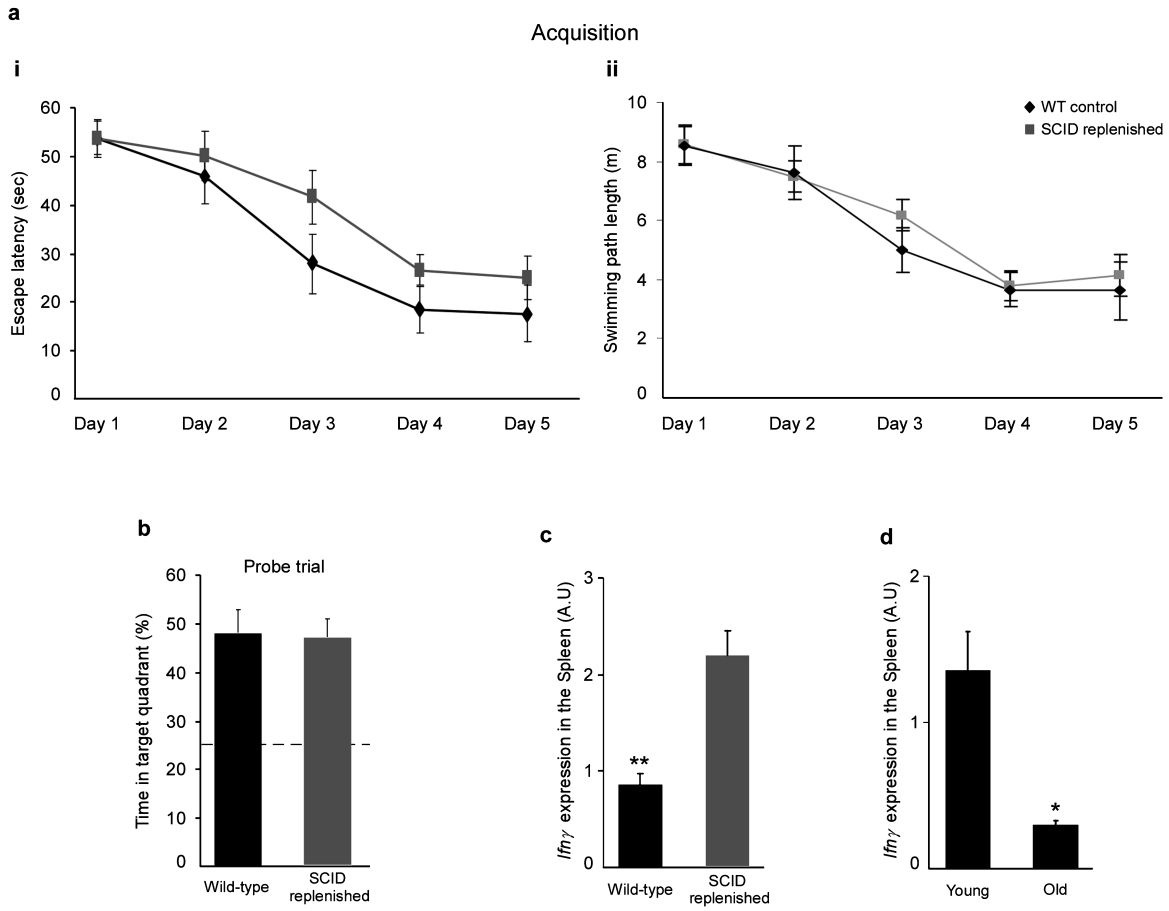

FIG. 4. Peripheral immune cells derived from aged mice restored spatial memory performance in SCID mice. C57BL/6 SCID mice were injected intravenously with lymphocytes derived from aged (20 months old) mice, and tested in the Morris water maze. (**a**) Average escape latency (**i**) and path length (**ii**) during acquisition. Each point represents the average of four trials a day. No differences were found between the groups (two-way-repeated-measures ANOVA; escape latency, groups, $F_{1,10} = 2.95$, $p = 0.12$; days, $F_{4,40} = 20.85$, $p = 0.0001$; groups × days, $F_{4,40} = 0.68$, $P = 0.61$; path length, groups, $F_{1,10} = 0.36$, $p = 0.56$; days, $F_{4,40} = 17.99$, $p = 0.0001$; groups × days, $F_{4,40} = 0.26$, $p = 0.90$). (**b**) A probe test was performed 3 days after the last acquisition day. Bars represent performance in the probe trial, calculated as in Fig.1b. No difference was found between the two groups ($t_{10} = -0.22$, $p = 0.83$, $n = 7$ and $n = 5$ for reconstituted SCID and wild-type mice, respectively). (**c**) Q-PCR analysis showing higher levels of *Ifn-*γ in the spleens of reconstituted SCID mice compared to the wild-type controls ($t_{13} = 3.46$, $^{**}p = 0.0042$). *Ifn-*γ levels tested in two nontreated SCID mice were lower than the wild-type (0.11 ± 0.02). (**d**) Q-PCR analysis showing reduced levels of *Ifn-*γ in the spleen of C57BL/6 aged mice (22 months, $n = 4$), compared to young (5 months, $n = 3$) controls ($t_3 = -3.9$, $^{*}p = 0.03$). Values represent the specified expression ratios in arbitrary units (A.U.), normalized against GAPDH in the same samples. Error bars represent SEM. Error bars represent standard error of the mean (SEM).

lowing the completion of the behavioral tests revealed that the levels of the *Ifn-γ* mRNA in the spleens of the SCID mice were almost nondetectable, whereas the *Ifn-γ* mRNA expression in the spleens of the SCID animals that received the aged lymphocytes was high and even higher than in wild-type mice (Fig. 4c). Importantly, under these conditions, spleen cells taken from old mice showed low levels of *Ifn-γ* expression (Fig. 4d) compared to young mice. According to these results, aged lymphocytes maintain, at least in part, the potential to support spatial memory performance. It is possible that the lymphopenic conditions of the SCID mice enabled this potential to be manifested. These results suggested that overcoming immune suppression in aged animals might be sufficient to improve their memory capacity. Therefore, we created in the aged animals the same lymphopenic conditions, as present in the SCID mice.

Lymphopenic conditions in the aged animals were created by total body irradiation, followed by the transfer of aged-matched homologous bone marrow cells. The bone marrow was shown to contain the repertoire of memory T cells accumulated throughout life.[18,19] Therefore, we assumed that in the aged animals, homeostatic-driven proliferation would result in expansion of the T cells derived from the transplanted bone marrow rather than from maturation of autologous naïve T cells, which are scarce due to age-related thymus involution.[20,21] This approach is used in cancer immunotherapy[15] and was also shown to generate neuroprotection.[16,22] Aged mice (15 months old) were lethally irradiated, while shielding the brain, and transplanted with bone marrow cells derived from identical syngeneic aged mice. Spatial memory ability was assessed 12 weeks following bone marrow transplantation by testing the behavior of the mice in the novel location recognition task. Performance in this hippocampal-dependent test is impaired in aged mice.[23] Moreover, unlike the Morris water maze, this test is not motor skill dependent. Old nontransplanted mice had no preference for exploration of the object in a novel location (median percent of preference time beyond that of the old location was 0.7), whereas the group of aged mice treated with the aged bone marrow showed a significant improvement in their spatial memory ability, manifested by their preference for exploration of the object in the novel location (median of 16.5%; $p < 0.05$, ANOVA), and did not differ significantly from the young mice (median of 17.0%; Fig 5a). In agreement with previous studies,[23] neither group of old mice differed from the young animals in their preferred exploration of a novel object (Fig. 5b). These results suggest that hippocampal-dependent memory deficit in aged mice does not depend solely on neurological factors; immune-based manipulations were sufficient to reverse some aspects of spatial memory deficits.

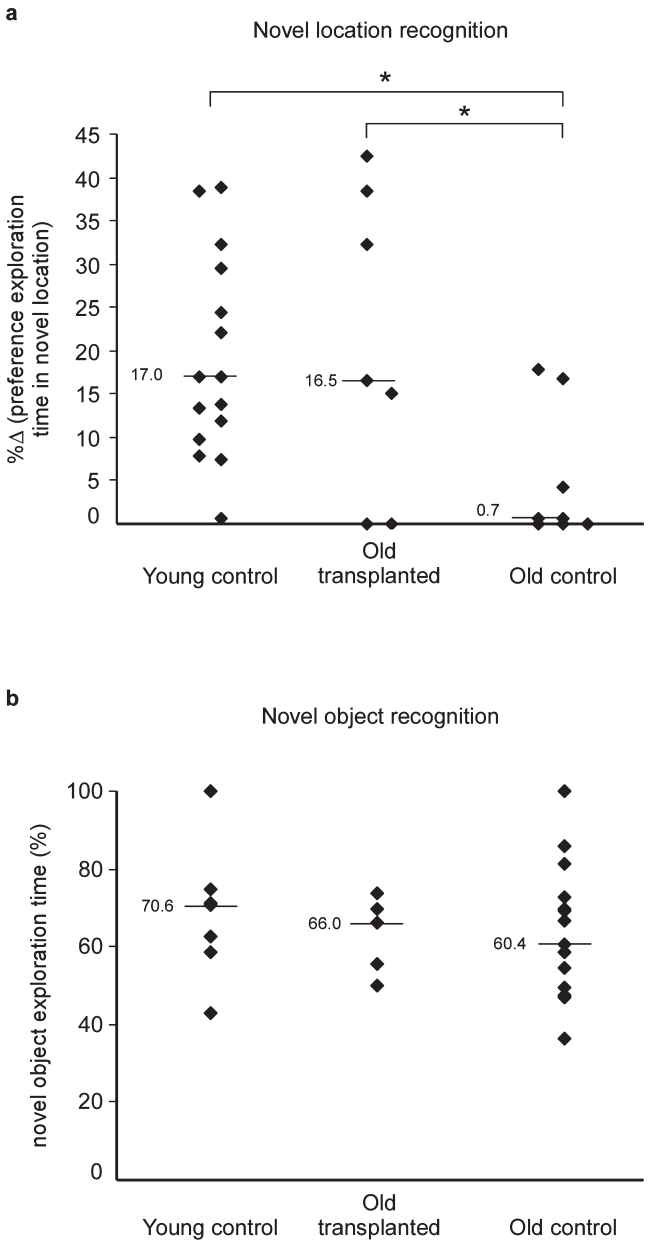

FIG. 5. Improved spatial memory performance in aged mice following bone marrow transplantation. Aged C57BL/6 mice (15 months old) were lethally irradiated and transplanted with bone marrow cells derived from mice of identical age. After 12 weeks, the transplanted mice as well as nontreated aged mice and young controls, on the same genetic background, were tested in the novel location recognition and novel object recognition tasks. (**a)** Novel location exploration was calculated as in Fig. 1c. Nontreated aged mice did not show any preference for exploration of the object in its novel location, whereas the performance of bone marrow-treated aged mice was comparable to young mice. A one-way ANOVA indicated a significant difference between treatment groups ($F_{2,27} = 4.07$, $p = 0.028$; $^*p < 0.05$; Fisher LSD *post hoc* analysis; $n = 15$ for wild-type young controls, $n = 8$ and $n = 7$ for aged-controls and aged-treated mice, respectively). Importantly, there were no differences in motor abilities among the two groups of aged mice, as indicated by the velocity and total distance moved that were measured during the test session (mean values ± SEM, velocity, 1.9 ± 0.2 cm/sec and 2.2 ± 0.3 cm/sec ($t_{13} = -1.03$, $p = 0.32$); distance, 11.5 ± 1.0 m and 13.4 ± 1.6 m ($t_{13} = -0.99$, $p = 0.34$) for aged controls and age-treated mice, respectively). (**b**) No differences were found in novel object recognition among the groups. (A one-way ANOVA, $F_{2,23} = 0.23$, $p = 0.8$.) Numbers represent median values for each group.

Discussion

The present study introduces the immune system as a novel player in functional brain aging and specifically in spatial memory. Aging in general may be viewed as the phenotype caused by damage accumulated in the body throughout life (as described in the "Disposable Soma" theory[24]). According to this view, failure in one of the body's mechanisms of maintenance and repair results in an increased rate of damage accumulation, leading to premature aging. In this study, we take this theory further by attributing a role in age-related memory loss to age-induced immune compromise. To our knowledge, a link between these two features of aging, immune function and memory, was never previously suggested.

A substantial decrease in immune responsiveness occurs with aging

Transplantation of SCID-derived bone marrow into wild-type mice at adulthood resulted in an exhaustion of the T cell compartment similar to the process that occurs with aging. Under these experimental conditions, we demonstrated a deficit in spatial memory. These results led us to consider that immune senescence is a causative factor in age-related memory loss. This linkage is supported by the reported observations that nearly every component of the immune system undergoes dramatic age-associated restructuring; however, the most prominent features of age-related immune senescence are involution of the thymus and alterations in thymocyte generation that results in a reduced number of peripheral naïve T cells.[4] In addition, age-related immune compromise has been associated with increased activity of $CD4^+CD25^+$ regulatory T cells (Treg)[25] and of myeloid-derived suppressor cells.[26] It is very likely that under the experimental paradigms used in the present study, of aged lymphocytes transferred to SCID mice and aged bone marrow cells transferred to lymphopenic aged-mice, homeostatic-driven proliferation took place.[16] Such conditions, possibly alleviated the aged immune system from suppression[15] and thereby enabled the activation of the existing T cell repertoire of the aged mice that contributed to memory restoration.

The choice of experimental conditions of the Morris water maze critically affects the ability to dissect out learning skills from memory abilities

A spatial memory deficit and its restoration were documented in the present study in C57BL/6 immune-compromised and aged mice using the Morris water maze and the novel location exploration task. There is an apparent discrepancy between the present study and our previous results.[3,7] Here, using an extended acquisition period and a larger pool size, we showed that immune-compromised mice had a deficit only in their spatial memory (probe trial), but not in the learning curve. In our early studies, the differences found between normal versus immune-compromised animals (on BALB/c background) at the acquisition stage made it impossible to distinguish between defects in learning versus memory defects. Specific memory impairments, however, are shown in the present study in immune-deficient mice on a C57BL/6 background and were verified also in the immune-deficient BALB/c mice (unpublished). Our ability to dissect out, in immune-compromised mice, spatial learning deficit from memory loss, one of the hallmarks of aging, further supports our contention regarding the relevance of immune senescence to aging.

Peripheral immunity affects expression of genes encoding for proteins associated with synaptic activity

The current study demonstrates that the lack or the loss of immune activity results in premature spatial memory decline, which we attribute, at least partially, to immune regulation of genes that are involved in presynaptic activity (*Syt10* and *Cplx2*)[11,27] and of *Igf1*.[12] Previous studies carried out by our group showed that the peripheral immune system is also involved in regulation of brain-derived neurotrophic factor (BDNF) levels and in stem/progenitor cell fate decisions in the healthy adult brain.[3] Those findings suggest additional mechanisms through which immune cells may support normal cognitive function.[28,29] In addition, we have found in immune-deficient mice a reduced ability to cope with stressful conditions and with depression.[30] Moreover, boosting peripheral immunity enhanced the ability of normal mice to cope with mental threats.[31] Those findings provide additional support for the role of the immune system in normal brain function. Our gene array analysis identified an additional gene, Glyoxalase-1, whose increased expression in the brain of young immune-deficient mice suggests premature damage accumulation as a result of immune deficiency. Glyoxalase-1 is a member of the glyoxalase detoxification mechanism, which fights against the accumulation of advanced glycation end products (AGEs).[32] AGE accumulation is a hallmark of normal brain aging and is part of the pathology in neurodegenerative diseases, including Alzheimer disease and diabetes.[33,34]

Inflammatory processes in the aging brain: A possible role for peripheral immunity in regulating microglial activity

Studies over the last decade have suggested that activated microglia, the resident innate immune cells in the central nervous system, can be either beneficial or detrimental to the brain, depending on the phenotype that they acquire.[35,36] In the diseased or injured brain, activated microglia produce a local inflammatory response that is often detrimental. Recent studies have shown, however, that infiltrating blood-borne monocytes can regulate such inflammatory microglia and thereby restore homeostasis and allow repair.[37,38] Such monocyte recruitment is increased by T cell-based vaccination.[39]

A local inflammatory response in the CNS is often associated with age-related cognitive deterioration.[40–42] Accordingly, boosting of T cell levels or reducing suppressor cell activity might enable mobilization of blood-borne monocytes and regain homeostasis.

In summary, our findings suggest that age-related memory loss may be reversible, at least to some extent, by a non-neurological intervention via manipulating the immune system. Our results also provide a possible explanation as to how physical activity alleviates some symptoms of brain aging,[43,44] possibly by increasing accessibility of the relevant immune components to the brain.[45–47] Thus, our results suggest that boosting of the specific immunity needed for CNS

plasticity[3,20,48,49] may provide a novel basis for developing new therapies to mitigate age-related memory loss, a key burden on aging society.

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