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Aging-associated deficit in CCR7 is linked to worsened lymphatic function, cognition, neuroinflammation, and amyloid pathology

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Aging leads to a progressive deterioration of meningeal lymphatics and peripheral immunity, which may accelerate cognitive decline. We hypothesized that an age-related reduction in C-C chemokine receptor type 7 (CCR7)–dependent egress of immune cells through the lymphatic vasculature mediates some aspects of brain aging and potentially exacerbates cognitive decline and Alzheimer’s disease–like amyloid (Aβ) pathology. We report a reduction in CCR7 expression by meningeal T cells in old mice that is linked to increased effector and regulatory T cells. Hematopoietic CCR7 deficiency mimicked the aging-associated changes in meningeal T cells and led to reduced lymphatic influx and cognitive impairment. Deletion of CCR7 in 5xFAD transgenic mice resulted in deleterious neurovascular and microglial activation, along with increased Aβ deposition in the brain. Treating old mice with anti-CD25 antibodies alleviated the exacerbated meningeal regulatory T cell response and improved cognitive function, highlighting the therapeutic potential of modulating meningeal immunity to fine-tune brain function in aging and in neurodegenerative diseases.

INTRODUCTION

Aging-related neurological disorders are rapidly becoming a major financial burden on health care worldwide. Alzheimer’s disease (AD) is the most prevalent aging-associated dementia, accounting for 60 to 80% of all dementia cases and affecting close to half of the elderly population over the age of 85 (1, 5). AD is characterized by severe behavioral deficits, particularly in cognitive faculties, whose underlying pathophysiological mechanisms are poorly understood and lack effective treatments (3–5). Accumulating evidence over the past decade has shown a close association between changes in immune system and the etiology and progression of AD (6–11). Microglia, the brain-resident immune cells, have been extensively studied and seem to play a central role in modulating AD pathology (11–15). Less attention has been focused, however, on changes in the adaptive immune response at the brain-meningeal border in aging and in AD.

The meninges, which ensheathe the brain, comprise a unique immune interface, harboring a diverse immune cell population that plays an essential role in maintaining brain homeostasis (16–18) and in fine-tuning processes such as neuroinflammation, tissue repair, and neuronal activity (19–25). Notably, different behavioral aspects such as cognition, sociability, and anxiety are modulated by meningeal T cell–derived cytokines that signal directly to their cognate receptors expressed on neurons (19, 26–28). The brain meninges also harbor bona fide lymphatic vessels that constantly drain molecular solutes from the cerebrospinal fluid (CSF) into the cervical lymph nodes (23, 30, 31). Aging in mice was recently shown to induce a deleterious loss of meningeal lymphatic coverage and drainage capacity, which is closely linked to cognitive decline (28). Ablation of the meningeal lymphatic vasculature in adult mice resulted in deficient clearance of brain solutes through the lymphatic system, as well as cognitive impairment and accumulation of amyloid (Aβ) in the brains of familial AD transgenic mice (28). Besides draining CSF, the lymphatic vasculature also regulates the immune response in the brain meninges (17, 53). Notably, it was shown that meningeal immune cell egress is mediated by C-C chemokine receptor type 7 (CCR7) expression and that ablation of meningeal lymphatic vessels in a model of neuroinflammation results in altered activation of T cells in the cervical lymph nodes (23).

Aging induces marked changes in the immune system (18, 32, 33). Moreover, the role of adaptive immune cells in AD was emphasized by reports showing altered AD-related Aβ brain pathology in immunodeficient mouse models (31, 55). Little is known, however, about the effects of aging on meningeal immunity and whether changes in meningeal immunity underlie the observed deficient clearance of brain waste and the build-up of Aβ in AD (17, 56–58). Here, in exploring the meningeal immune profiles of old mice, we observed a reduction in CCR7 expression by T cells. To investigate a potential link between this decreased CCR7 expression in immune cells and brain dysfunction, we examined the changes in meningeal immunity, cognition, lymphatic function, and brain single-cell transcriptomic profile in CCR7-deficient mice. We also provide evidence showing that decreased CCR7 expression affects brain Aβ pathology and cognitive function in a mouse model of familial AD and that normalization of the surrogate regulatory T cells (Treg) in the cerebral meninges has beneficial effects.
analysis of the T cell response in mouse meningeal preparations (composed mostly of dural and arachnoid layers) revealed a significantly larger number of T cells (NK1.1 TCRβ+) in old mice (24 to 25 months of age) than in adult (2 to 3-month-old) mice (Fig. 1A to 6), confirming previously published findings (14). Assessment of forkhead box P3 (FOXP3) expression by meningeal leukocytes revealed a significant increase in the frequency and number of CD4+FOXP3+ Tregs in the old mice (Fig. 1D and fig. S1A). Assessment of the brain-draining deep cervical lymph nodes (dCLNs) revealed decreased frequency of T cells and an increase in frequency of the Tregs in old mice (Fig. 1E to H). Together, this translated into an overall significant decrease in the number of CD4+FOXP3+ effector T cells (Fig. 1B). Analysis of blood and liver samples revealed no differences in total CD45+ cells or in CD4+FOXP3+ Tregs between adult and old mice (Fig. S1, C to J).

We have previously shown that impaired signaling through CCR7 results in accumulation of T cells in the brain meninges (23). To find out whether altered CCR7 expression could explain the
increase in T cell numbers observed in the meninges of old mice, we
performed direct ex vivo staining of CCR7 (fig. S1K) on meningeal
immune cells from mice at the ages of 4 months (adult) and 25 months
(old). A smaller proportion of CCR7-expressing T cells, including
CD4+FOXP3+CCR7high Tregs, was observed in meninges of the old
mice than of the adult mice (fig. 1, A and B). Decreased proportions of
CCR7-expressing effector T cells and Tregs were also observed in the
dCLNs of old mice (fig. 1, C and D). These data indicate that old mice exhibit T cell accumula-
tion in the meninges and decreased T cell retention in the dCLNs,
possibly owing to their lack of CCR7 and their impaired migration
capacity.

Next, we used CCR7GFP reporter mice to evaluate CCR7 ex-
pression levels in adult (3-month-old) and middle-aged (12- to
14-month-old) mice. Analysis of the brain cortex, choroid plexus,
and meninges of adult CCR7GFP reporter mice by flow cytometry
(fig. S2, A to C) showed that most of the brain-associated CCR7high
leukocytes are predominantly found in the meninges (fig. S2B).
Moreover, a significantly higher frequency of CCR7high leukocytes
was observed in the dCLNs than in the blood and liver (fig. S2C),
again underscoring the importance of CCR7 as a mediator of leuko-
cyte egress through lymphatic vessels from the meninges into the
dCLNs. We also found that most of the CCR7high leukocytes in the
meninges were also T cell receptor positive (TCR+; ~83% of total
CD4+CD25+ T cells expressed high levels of CCR7 (fig. S2F). Ap-
proximately 96% of CCR7high leukocytes in the dCLNs and 95% in the
blood were TCR+ (fig. S2, D and E), and that ~27% of meningeal
CD4+CD25+ T cells expressed high levels of CCR7 (fig. S2F). These
findings underscore the importance of CCR7 as a mediator of leuko-
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Hematopoietic CCR7 deficiency hinders spatial memory
and glymphatic function
T cells participate in the modulation of neuronal activity and higher
cognitive functions (19, 50, 51). In view of the abnormal T cell
response due to CCR7 deficiency, we compared the performance of
5- to 7-month-old CCR7−/− mice and their age-matched WT litter-
mates in different behavioral tests. In the open-field test, both groups
showed comparable values in terms of total distance traveled, velocity,
and time spent in the center of the arena, which indicated similar
exploratory activity and anxiety-like behavior (fig. S6, A and C).
Equivalent performances in the open field were also observed after
(WT or CCR7−/−) BMT (fig. S6, D and F). However, both CCR7−/−
mice (fig. S6B and H) and WT mice that had received CCR7−/− bone
marrow (at 4 months of age; fig. S6B and H) performed worse in the
novel location recognition and Morris water maze (MWM) tests
than their respective controls. These results are in line with previous
reports showing deficits displayed by CCR7−/− mice in the
Barnes maze test (37), reinforcing the notion that impaired CCR7-dep-
dependent immune cell egress is associated with worse cognitive
function.

Reduced meningeal lymphatic drainage has been linked to both
aging-related cognitive decline and impaired recirculation of CSF
through the brain via the glymphatic system (28). On the basis of
these findings, we evaluated lymphatic drainage in CCR7−/− and
WT mice. Lymphatic markers, such as LYVE1, were expressed in
the brain meninges and meningeal lymphatic vessels (fig. S7, A and
B), and a comparable number of vessels were observed in CCR7−/− and
WT mice (fig. S7, C and D). These data indicate that lymphatic
vascularization in the brain is not affected by CCR7 deficiency.
Fig. 2. CCR7 deficiency in hematopoietic cells mimics the aging-related dysregulated meningeal T cell response. (A to F) Bone marrow (BM) from 2-month-old WT or CCR7-deficient (CCR7−/−) mice was transferred into irradiated (head-covered) WT recipients (6 weeks old). Immune response and behavior were assessed 10 weeks later. Quantification of CD45+ZA− cell number, representative flow cytometry dot plots, and quantification of DN, CD4+, and CD8+ T cell numbers in the (A to C) meninges and (D to F) dCLNs. Data are presented as means ± SEM; n = 5 per group; two-tailed unpaired Student’s t test in (A) and (D); two-way ANOVA with Sidak’s multiple comparisons test in (C) and (F). (G) t-distributed stochastic neighbor embedding–based visualization (viSNE) plots showing unsupervised clustering profile of subpopulations of CD45+ live immune cells. NK cells, natural killer cells; RBCs, red blood cells. (H) Volcano plot with change in frequency (in percentage) of subpopulations of meningeal leukocytes in CCR7−/− mice (relative to WT, n = 5 per group). Individual data points represent the mean for each leukocyte population; multiple two-tailed unpaired Student’s t tests with two-stage step-up method of Benjamini, Krieger, and Yekutieli and false discovery rate (FDR) (Q) = 0.05. (I) viSNE plots showing clustering of sub-