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Aging-associated deficit in CCR7 is linked to worsened glymphatic function, cognition, neuroinflammation, and β-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 brain β-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 glymphatic 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, 2). 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 neuroimmune-related mechanisms 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 ensheath the brain, comprise a unique neuroimmune 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, 25, 26). 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, 26, 27). Aging in mice was recently shown

to induce a deleterious loss of meningeal lymphatic coverage and drainage capacity, which is closely associated with cognitive decline (28). Ablation of the meningeal lymphatic vasculature in adult mice resulted in deficient clearance of brain solutes through the glymphatic system, as well as cognitive impairment and accumulation of β-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, 23). 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, 29, 30). 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, 32). 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, 18, 33, 34). 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, glymphatic 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 nor-

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. 1, A to C), 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⁺ T_{regs} 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 T_{regs} in old mice (Fig. 1, E to H). Together, this translated into an overall significant decrease in the number of CD4⁺FOXP3⁻ effector T cells (fig. S1B). Analysis of blood and liver samples revealed no differences in total CD45⁺ cells or in CD4⁺FOXP3⁺ T_{regs} 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
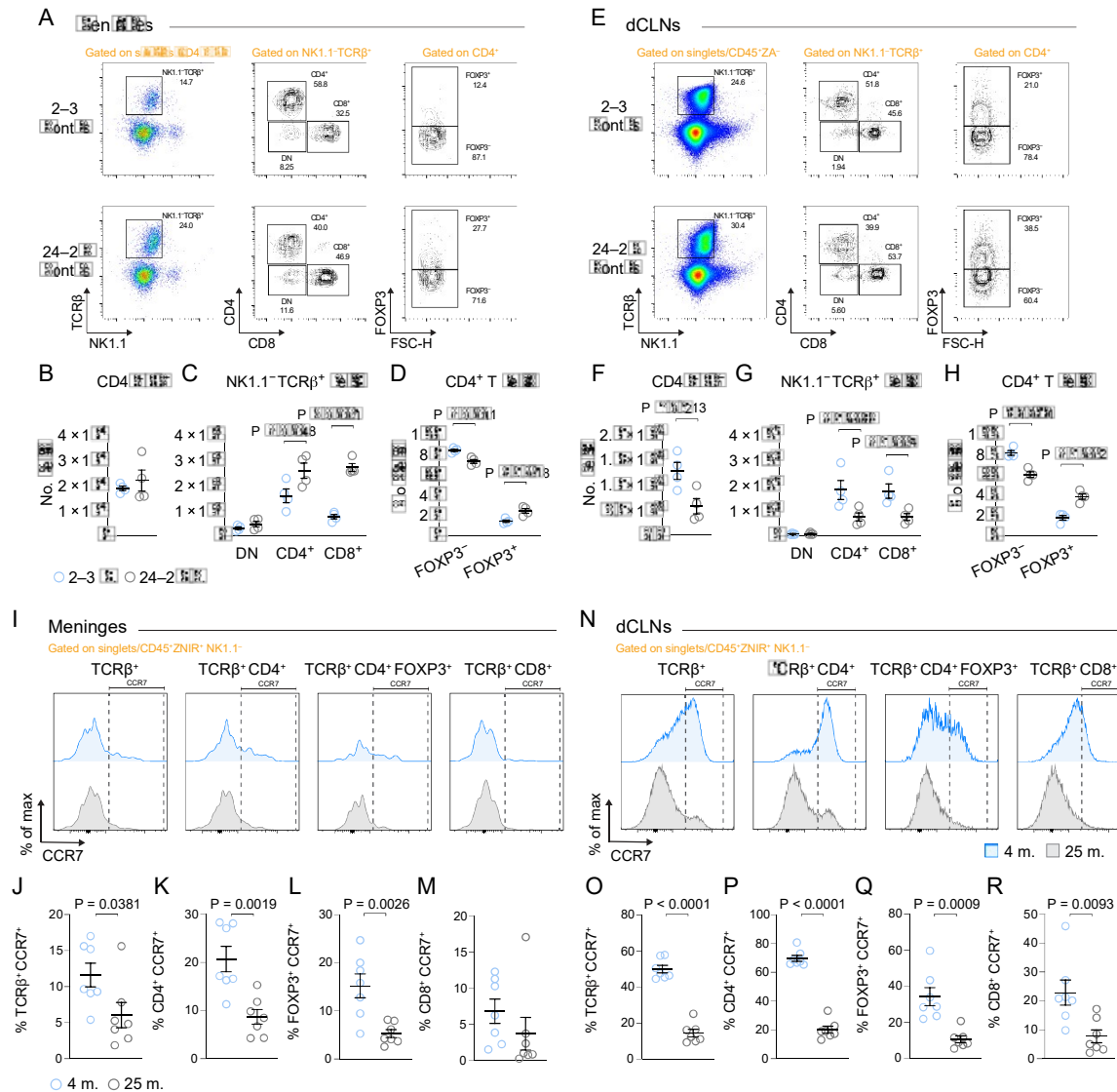

Fig. 1. Aging leads to abnormal T_{reg} response and reduced CCR7⁺ T cell frequency in the brain meninges and draining lymph nodes. (A) Representative flow cytometry dot and contour plots showing the gating strategies used to determine NK1.1⁻TCRβ⁺ cells, CD4⁺, CD8⁺, DN T cells, and FOXP3⁻ or FOXP3⁺ CD4 T cells in the me-

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 $CD4^+FOXP3^+CCR7^+$ T_{regs}, was observed in meninges of the old mice than of the adult mice (Fig. 1, L to M, and fig. S1, L to O). Decreased proportions of CCR7-expressing effector T cells and T_{regs} were also observed in the dCLNs of old mice (Fig. 1, N to R, and fig. S1, P to S). These data indicate that old mice exhibit T cell accumulation 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 $CCR7^{GFP}$ reporter mice to evaluate CCR7 expression 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 $CCR7^{GFP}$ reporter mice by flow cytometry (fig. S2, A to C) showed that most of the brain-associated $CCR7^{high}$ leukocytes are predominantly found in the meninges (fig. S2B). Moreover, a significantly higher frequency of $CCR7^{high}$ leukocytes was observed in the dCLNs than in the blood and liver (fig. S2C), again underscoring the importance of CCR7 as a mediator of leukocyte egress through lymphatic vessels from the meninges into the dCLNs. We also found that most of the $CCR7^{high}$ leukocytes in the meninges were also T cell receptor β positive (TCRβ; ~83% of total $CD45^+CCR7^{high}$; fig. S2, D and E) and that ~27% of meningeal $CD4^+CD25^+$ T cells expressed high levels of CCR7 (fig. S2F). Approximately 96% of $CCR7^{high}$ leukocytes in the dCLNs and 95% in the blood were TCRβ (fig. S2, D and E), and more than half of the $CD4^+CD25^+$ T cells in the dCLNs expressed high levels of CCR7 (fig. S2F). Thus, consistent with our ex vivo staining data, the middle-aged $CCR7^{GFP}$ reporter mice showed a significant decrease in CCR7-expressing TCRβ T cells, relative to their adult counterparts in the meninges and dCLNs (fig. S2, G and H), but not in the blood or liver (fig. S2, I and J).

Together, these results point to a heightened T_{reg} response in the brain meninges and draining dCLNs of old mice, with concomitant reduction in $CCR7^{high}$ T cells. In line with our data, previous studies have reported an increased frequency of $CD4^+FOXP3^+$ T_{regs} in the spleen and peripheral lymph nodes of old mice relative to their younger counterparts (35).

CCR7 deficiency boosts meningeal T_{regs} in adult mice

To find out whether a decrease in CCR7 expression in the hematopoietic compartment would mimic the observed aging-related effects on meningeal immunity, we irradiated adult wild-type (WT) mice (while covering their heads with a lead shield to preserve brain-resident immune cells) and then carried out adoptive transfer of bone marrow cells isolated from WT or CCR7-deficient ($CCR7^{-/-}$) donor mice (see fig. S3A for more experimental details). As observed earlier in old mice, mice that received $CCR7^{-/-}$ bone marrow also showed increased numbers of $CD4^+$ (and $CD8^+$) T cells in the

enabled us to identify 21 clusters (and a cluster of undefined cells; Fig. 3G and fig. S4A), of which only cells of the CD4 and CD8 clusters were significantly increased in $CCR7^{-/-}$ mice (Fig. 3H). This increased number of meningeal T cells in the $CCR7^{-/-}$ group was associated with a decrease in the number of cells in the macrophage 2 cluster (Fig. 3H). Upon closer analysis of activation markers and transcription factor expression on cells from the three identified $CD3^+TCR\beta$ T cell clusters (CD4, CD8, and double-negative T cells; fig. S4B), we observed an increased frequency of $CD8^+$T-bet^{low} T cells and of $CD4^+FOXP3^+$ T_{regs} in the meninges of $CCR7^{-/-}$ mice (Fig. 3, I and J). The increased frequency of meningeal T_{regs} in the $CCR7^{-/-}$ group was accompanied by a decrease in all T-bet^{high} T cell subclusters (Fig. 3, I and J). Flow cytometric analysis of ex vivo–stimulated meningeal T cells confirmed the increase in frequency of $CD4^+FOXP3^+$ T_{regs} in $CCR7^{-/-}$ mice and a concomitant decrease in frequency of interferon-γ (IFN-γ)–producing CD4 T cells (fig. S4, C to F). Both CD4 and CD8 meningeal T cells from the $CCR7^{-/-}$ group showed a trend toward decreased IFN-γ production relative to the WT control (fig. S4, G to I). The increase in T_{regs} in the $CCR7^{-/-}$ group was evident in the meninges and dCLNs but was only minor in the liver and was not detected in the blood (fig. S5). Together, these results show that CCR7 deficiency (either constitutive or upon BMT) leads to an aging-like dysregulated T cell response, characterized by decreased T-bet^{high} and IFN-γ–producing CD4 T cells and an abnormal increase in T_{regs} in the brain meninges and dCLNs.

Hematopoietic CCR7 deficiency hinders spatial memory and glymphatic function

T cells participate in the modulation of neuronal activity and higher cognitive functions (19, 32, 35, 36). 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 littermates 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 to C). Equivalent performances in the open field were also observed after (WT or $CCR7^{-/-}$) BMT (fig. S6, D to F). However, both $CCR7^{-/-}$ mice (Fig. 4, A to E) and WT mice that had received $CCR7^{-/-}$ bone marrow (at 4 months of age; Fig. 4, F to J) performed worse in the novel location recognition and Morris water maze (MWM) tests than their respective controls. These results are in line with previously reported learning deficits displayed by $CCR7^{-/-}$ mice in the Barnes maze test (37), reinforcing the notion that impaired CCR7-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 that finding, we next examined both the glymphatic

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-