Resident Memory-like CD8⁺ T Cells Are Involved in Chronic Inflammatory and Neurodegenerative Diseases in the CNS

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Abstract

Background and Objectives
Resident memory T (Trm) cells are a unique population that can survive and function in a compartmentalized tissue with inflammatory potential. We aim to investigate the alteration of Trm population in acute/chronic inflammatory and neurodegenerative diseases in the CNS.

Methods
The frequencies of CD4⁺ and CD8⁺ T cells expressing both CD69 and CD103, the markers for Trm cells, were quantified in the peripheral blood and CSF (n = 80 and 44, respectively) in a cross-sectional manner. The transcriptional profile of Trm-like population in the CSF was further analyzed using a public single-cell dataset.

Results
The frequency of CD69⁺CD103⁺CD8⁺ T cells was strikingly higher in the CSF than in the peripheral blood (among memory fraction, 13.5% vs 0.11%, difference (mean [SE]): 13.4% [2.9]). This CD69⁺CD103⁺CD8⁺ T-cell population was increased in the CSF from patients with chronic inflammatory diseases including multiple sclerosis and with neurodegenerative diseases such as Parkinson disease and Alzheimer disease compared with controls (11.5%, 13.0%, 8.1% vs 2.9%, respectively). By contrast, the frequency was not altered in acute inflammatory conditions in the CNS (4.0%). Single-cell RNAseq analysis confirmed Trm signature in CD69⁺CD103⁺CD8⁺ T cells in the CSF, supporting their Trm-like phenotype, which was not clear in controls.

Discussion
Collectively, an increase in CD69⁺CD103⁺CD8⁺ Trm-like population in the CSF is related with both chronic neuroinflammatory and some neurodegenerative diseases in the CNS, suggesting a partially shared pathology in these diseases.

Introduction
Inflammatory CD4⁺ and CD8⁺ T cells play a pivotal role in autoimmune diseases of the CNS. It has been recently reported that T cells in the CNS of patients with multiple sclerosis (MS) have distinct transcriptomic characteristics, including expressions of CD69 and CD103, which are markers of resident memory population.¹,² There tissue-resident memory-like CD8⁺ T (Trm-like CD8⁺) cells, which populate active inflammatory lesions of patients with MS, showed signs of reactivation and...
Glossary

AD = Alzheimer disease; ALS = amyotrophic lateral sclerosis; MS = multiple sclerosis; NMOSD = neuromyelitis optica spectrum disorder; PBMC = peripheral blood mononuclear cells; PD = Parkinson disease; Trm = resident memory T.

cytotoxicity. Consistently, clonally expanded CD8+ T cells in the CSF of patients with MS showed Trm-like transcriptome compared with nonexpanded CD8+ T cells.

Clonally expanded CD8+ T cells are also found in the CSF of patients with Alzheimer disease (AD). The clones are characterized by increased gene expressions of cytotoxic molecules, such as GZMA. GZMA+CD8+ T cells are prevalent in the hippocampi of patients, suggesting their pathologic role. Consistently, T-cell infiltration in the hippocampi was found to correlate with AD pathology. Inflammatory T cells are also known to infiltrate in neurogenic niches in the aged brain, thus inhibiting proliferation of neural stem cells. Another study showed CD69+CD103+CD8+ Trm-like cells with polyfunctionality in the brains from patients with Parkinson disease (PD). However, it is not yet known how Trm-like cells in the CNS compartment are numerically and functionally altered in various neurologic diseases.

In this study, we show that CD69+CD103+ Trm-like CD4+ and CD8+ cells are more prevalent in the CSF than in the peripheral blood. Their frequencies in the peripheral blood are not altered in patients with MS nor neuromyelitis optica spectrum disorder (NMOSD) compared with controls. By contrast, CD69+CD103+CD8+ T cells are increased in the CSF from patients with chronic neuroinflammatory diseases, including MS, compared with those in controls. Notably, this cell type is similarly increased in PD and AD. We further analyzed their transcriptional profile at a single-cell level using a public dataset, confirming classical Trm signature in CD69+CD103+CD8+ T cells. Collectively, the increase of this CD8+ Trm-like cell type is related not only with neuroinflammatory but also with some neurodegenerative pathologies, suggesting their involvement in a disease process shared by these conditions.

Method

Participants

Demographics of the patients and controls are described in eTable 1 (links.lww.com/NXI/A938). All patients were treated at National Center Hospital of Neurology and Psychiatry or Kyoto University Hospital, Japan, from 2014 to 2020. Patients were consecutively chosen whenever the experiments could be performed. The study size was not predetermined. Attending neurologists diagnosed patients according to McDonald criteria 2010 for MS, international consensus diagnostic criteria for NMOSD, MDS clinical diagnostic criteria for PD, the 2011 guidelines in the National Institute on Aging–Alzheimer’s Association for probable AD and mild cognitive impairment due to AD, and the combination of Awaji diagnostic algorithm and clinical course for amyotrophic lateral sclerosis (ALS). Patients with MS were categorized as in relapse state when they experienced a new or worsening neurologic deficit lasting more than 24 hours within the immediate 4 weeks before the collection of the sample. Details of the participants are described in eTable 1, A and B.

Standard Protocol Approvals, Registrations, and Patient Consents

The Ethics Committee of National Center of Neurology and Psychiatry and Kyoto University Hospital approved the study protocol according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Cell Preparation and Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation, using Ficoll-Paque PLUS (GE Healthcare Bioscience, ON, Canada). PBMCs and cells from the CSF were stained for cell surface antigens. The antibodies and isotype controls used in this study were as follows: FITC-anti-CD3 (UCHT1) (Beckman Coulter, CA), PerCP-Cy5.5-anti-CD4 (UCHT1), PE-anti-CD11a (TS2/4), BV510-anti-CCR7 (G043H7), APC-anti-CD69 (FN50), PerCP-Cy5.5-anti-CD69 (FN50), PE-anti-CD103 (Ber-Act8), BV421-anti-CD103 (Ber-Act8), APC-Cy7-anti-CD8a (RPA-T8), FITC-anti-CD4 (RPA-T4) (BioLegend, CA), V500-anti-CD4 (RPA-T4), PE-Cy7-anti-CD45RA (L48) (BD Biosciences, NJ) Cells were analyzed in the CSF and sorted using FACS Canto II (BD Biosciences) and FACS Aria II (BD Biosciences). Data were analyzed with FlowJo software (BD Biosciences). At least 50 cells of CD8+ T cells were analyzed in the CSF of each participant.

Single-Cell RNA Sequencing

The single-cell RNA sequencing data of CD8+ T cells in the peripheral blood and CSF from patients with MS were obtained from GEO repository under GSE127969, which were analyzed with BBrowser (BioTuring) (eTable 2, links.lww.com/NXI/A939).

Statistical Analysis

Data were analyzed with Prism software (GraphPad Software, CA) unless otherwise indicated. An unpaired or a paired t test was used to compare data from 2 groups. One-way ANOVA with the Holm-Sidak multiple comparison test was used to compare data from more than 2 groups. The Pearson analysis was used to evaluate correlations. Differences were considered significant when p value was <0.05.

Data Availability

Anonymized data not published within this article will be made available by request from any qualified investigator.
Result

First, we evaluated the frequency of Trm-like cells in the peripheral blood of patients with MS and NMOSD, using the characteristic markers, CD69 and CD103. As expected, CD45RA− memory fraction was enriched in CD69+CD103+ double-positive (DP) cells compared with CD45RA+ fraction, both in CD4+ and CD8+ T cells (Figure 1, A–D, eFigure 1, links.lww.com/NXI/A935). The frequency of memory CD4+ or CD8+ T cells was not altered, except for subtle increase of memory CD4+ T cells during relapses in MS (eFigure 2, A and B, links.lww.com/NXI/A936). Because the frequencies of CD69+CD103+ DP fraction among memory CD4+ and CD8+ T cells were not altered by disease conditions (Figure 2, A and B), we focused on their presence in the CSF, surrounding brain parenchyma where the disease process is taking place. The transcriptional profile of T cells in the CSF is known to resemble that of T cells in the brain parenchyma in a mouse model of MS,13 which is also suggested in MS.14 The frequencies of CD69+CD103+ DP T cells among memory CD8+ T cells were strikingly higher in the CSF than in the peripheral blood, from 4 patients with MS and 1 with NMOSD (Figure 2, C and D). We observed a similar trend among memory CD4+ T cells (Figure 2D).

To investigate whether their frequencies are affected by diseases in the CNS, we recruited patients with acute and chronic inflammatory diseases, and neurodegenerative diseases such as PD, AD, and ALS. Most of CD4+ and CD8+ T cells in the CSF belonged to memory fraction irrespective of disease categories (eFigure 2, C and D, links.lww.com/NXI/A936). Notably, CD8+ Trm-like cells were significantly increased in chronic inflammatory diseases, PD, and AD compared with the control group (Figures 3A and 4A). All 4 patients in the MS* group were not in a relapse state (Figure 4A). On the contrary, the frequency was not altered in acute inflammatory conditions and ALS (Figure 4A). CD4+ Trm-like cells showed similar trend without significance (Figures 3B and 4B).

Because CD8+ T cells in the CSF contain CD45RA+ CCR7+ TEMRA population (eFigure 3, A and B, links.lww.com/NXI/A937), we compared the frequency of CD69+CD103+ fraction among TEMRA and CD45RA− populations. The CD69+CD103+ frequency was lower in CD8+ TEMRA cells (eFigure 3C), and not significantly different between various disease categories (eFigure 3D), though there was a similar trend as in CD45RA− CD8+ T cells (Figure 4A).
T-cell infiltration from the periphery during a short period after the onset might reduce CD8+ Trm-like cell frequency in acute inflammatory conditions because this population is rarely seen in the peripheral blood (Figure 2C,D). However, there was no significant correlation between CD8+ Trm-like cell frequency and total cell number in the CSF, suggesting this is not a major determinant of CD8+ Trm-like cell frequency in the CSF (Figure 4C). In addition, it cannot explain low frequency of CD8+ Trm-like cells in controls lacking substantial recruitment of peripheral T cells (Figure 4A,B). Although it was shown that inflammatory T cells infiltrate in neurogenic niches in aged brain, 6 we found no apparent effect of aging on Trm-like cells in the CSF (Figure 4D). Three patients with acute inflammatory diseases were followed up to approximately 80 days after the onset. All the patients were recovering during the follow-up period. The CD8+ Trm-like cell frequency slightly increased over time (Figure 5A), supporting their persistence in the local tissue. This was not the case for CD4+ Trm-like cells (Figure 5B). It needs further investigation to elucidate how long the increased frequency is sustained after the onset of acute inflammatory diseases and how their antigen specificity is involved in this long-term process.

Finally, we evaluated the characteristics of these CD69+CD103+ DP CD8+ T cells in the CSF using a public single-cell RNA sequencing dataset of immune cells in patients with MS. 3 We included 3 CD8+ T-cell populations, CD69+CD103+ DP from the CSF, non-DP from the CSF, and total from the peripheral blood, both in MS and noninflammatory control (intracranial idiopathic hypertension). In patients with MS, DP CD8+ T cells showed decreased expressions of CCR7, SELL, S1PR1, and KLRG1, suggesting their effector phenotype and tissue residency (Figure 5C). 15,16 DP CD8+ T cells were also characterized by high expressions of PRDM1 and ZNF683, the core transcription factors of Trm cells (Figure 5C). 17 In addition, CD44, CXCR6, and CCL5 were upregulated in DP CD8+ T cells, further supporting Trm phenotype (Figure 5C). CD44 and CXCR6 are among the core Trm signature in human. 15,18 CCL5 is known to be expressed by Trm cells in the postinfectious brain, which creates permissive environment for subsequent local autoimmunity. 19 By contrast, such gene expression pattern was not clearly observed in controls (Figure 5D). SIPRI, which is used for egress of T cells out of the tissue into the circulation, was rather upregulated in DP and non-DP CD8+ T cells in the CSF compared with peripheral CD8+ T cells in controls (Figure 5D). These data suggest that CD69+CD103+ DP CD8+ T cells in MS have phenotype closer to Trm than in HC.

Discussion
The compartmentalized immune reaction inside the CNS is now regarded as an important contributor to the pathologies of both
autoimmune diseases and neurodegenerative diseases. Among diverse immune cell types, resident T cells in the CNS are attracting increasing attention. Migration of peripherally activated T cells causes various autoimmune diseases, such as relapsing-remitting MS (RRMS), where T-cell targeting therapy shows substantial efficacy. On the contrary, mechanism underlying progressive MS seems to be different, which is suggested by the failure of the same treatment strategy as for RRMS. CD69+CD103+CD8+ Trm-like cells are found in the inflamed foci of the brain of patients with MS and multifunctional upon activation. Because Trm cells are usually not replenished from the periphery, where current T cell–targeted therapy can reach, this population potentially damages the brain in drug-privileged environment. This speculation is supported by a recent study that showed persisting CD8+ Trm cells in the brain contribute to CNS inflammation and progressive loss of neurons independently of circulating CD8+ T cells.20,21

Trm-like cells are suggested to be also involved in the pathology of PD. CD8+ T cells are increased in the substantia nigra of patients with PD, equipped with cytolytic enzymes and proinflammatory cytokines, and their density positively correlates with neuronal death.22 Of interest, approximately a half of these CD8+ T cells express CD103.22 On the contrary, no difference was found regarding the infiltration of CD4+ T cells,22 which is in line with our data (Figure 4B). In patients with AD, clonally expanded CD8+ T cells are found in the CSF, with potential pathogenicity.4 The clonal expansion of CD4+ T cells was not as much evident as CD8+ T cells,4 which is possibly consistent with our data (Figure 4B). It was shown that T-cell infiltration drives neurodegeneration in a mouse model of AD.23 Despite all these data, it was not yet known how Trm-like cells in the CNS compartment are numerically and functionally altered in various neurologic diseases.

We found that CD8+ Trm-like cells are abundant in the CSF not only in patients with chronic inflammatory diseases including MS but also in the patients with neurodegenerative diseases such as PD and AD, compared with those in controls (Figures 3A and 4A). This population is rarely seen in the peripheral blood, highlighting importance of evaluation of the CSF, where T-cell phenotype is similar to that in the CNS parenchyma.13,14 The function of Trm cells has been well studied in the context of local infection. Trm cells persist after viral infection in the CNS, rapidly respond to the second challenge, and prevent fatal consequence, even without help from circulating memory CD8+ T cells.24 Consistently, CD69+CD103+CD8+ Tm-like cells in the human brain were shown to secrete multiple inflammatory cytokines.7 We found an accumulation of such Trm-like CD8+ T cells in chronic neuroinflammatory and some neurodegenerative diseases, suggesting partly shared pathomechanism (Figures 3A and 4A). CD69+CD103+CD8+ T cells showed transcriptomic signature characteristic of tissue residency in patients with MS, which supports their Trm-like function (Figure 5C). Of interest, this pattern was not observed in controls (Figure 5D). Relatively maintained expression of S1PR1 in Trm-like cells in controls may indicate that this population is
less compartmentalized to the CNS in physiologic condition (Figure 5D) because they can sense a higher concentration of S1P in the peripheral circulation and exit toward it.\(^{25}\) It should be addressed in the future whether tissue residency–related gene expression is different across chronic inflammatory and neurodegenerative diseases in the CNS. It would be of interest to study whether the frequency and transcriptome of Trm-like cells correlate with disease severity or duration, for which the number of patients was not enough in the current study. Trm cells are also known to be transcriptionally different between active lesions and normal-appearing white matter in MS,\(^{26}\) which warrants lesion-specific analysis in other neurologic diseases. Furthermore, it would be interesting to study the interaction between Trm cells and other immune cell subsets, which may be feasible by deep analysis of immune cells in the CSF, including border-associated macrophages.\(^{27}\)

While CXCR6 is known to be a signature gene of Trm cells, CXCR6\(^+\)CD4\(^+\) T cells were reported as more pathogenic IFN-\(\gamma\)GM-CSF\(^+\)Th17 cells in experimental autoimmune encephalomyelitis, a mouse model of MS.\(^{28}\) CXCR6\(^+\)CD4\(^+\) T cells can infiltrate broader range of CNS parenchyma including gray matter in contrast to CXCR6\(^-\)CD4\(^+\) T cells,\(^{29}\) which is notable considering that gray matter lesions are closely correlated with severity and prognosis of MS.\(^{30,31}\) Recently, it was suggested that the recruitment of CD8\(^+\) T cells into the CSF is mediated through CXCR6-CXCL16 pathway in patients with AD.\(^{32}\) It warrants further investigation into whether this CXCR6-CXCL16 pathway is a shared mechanism of CD8\(^+\) T-cell infiltration among various neurologic diseases and whether CXCR6\(^+\)CD8\(^+\) T cells have more pathogenic role, considering a high expression of CXCR6 on Trm-like CD8\(^+\) T cells (Figure 5C).

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Figure 5 CD69⁺CD103⁺ DP CD8⁺ T Cells From Patients With MS Have a Phenotype Closer to Trm Compared With Those From HC

(A) Temporal alteration of the frequency of CD8⁺ Trm-like cells during the recovery period of acute inflammatory diseases. (B) Temporal alteration of the frequency of CD4⁺ Trm-like cells during the recovery period of acute inflammatory diseases. (C) Gene expressions in CD69⁺CD103⁺ DP CD8⁺ T cells in the CSF are analyzed using public single-cell RNA sequencing dataset. Three CD8⁺ T-cell populations are shown; CD69⁺CD103⁺ DP in the CSF, non-DP in the CSF, and total CD8⁺ T cells in the peripheral blood, from patients with MS. The sum of expressions for each gene in each condition are normalized to 1. (D) CD8⁺ T cells from patients with a noninflammatory condition (intracranial idiopathic hypertension) were analyzed in the same way as in (C). PB = peripheral blood.

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Disclosure
The authors report no relevant disclosures. Go to Neurology.org/NN for full disclosures.

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## Resident Memory-like CD8^+ T Cells Are Involved in Chronic Inflammatory and Neodegenerative Diseases in the CNS


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