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BRIEF COMMUNICATION

Elevated CNS inflammation in patients with preclinical Alzheimer's disease

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Alzheimer's disease (AD) is a progressive, neurodegenerative disease that may involve inflammatory responses in the central nervous system (CNS). Our objective was to determine whether patients with amnestic mild cognitive impairment (aMCI), a preclinical stage of AD, have inflammatory characteristics similar to patients with multiple sclerosis (MS), a known CNS inflammatory disease. The frequency of lymphocytes and levels of pro-inflammatory cytokines in the cerebrospinal fluid of aMCI patients was comparable to MS patients or patients at high risk to develop MS. Thus, brain inflammation occurs early at the preclinical stage of AD and may have an important role in pathology.

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Keywords: Alzheimer's disease; dementia; inflammation; leukocytes; T cells

INTRODUCTION

Previous studies of *post-mortem* brain tissue of patients with Alzheimer's disease (AD) have repeatedly shown evidence of brain inflammation, including the presence of activated microglia and astrocytes, T cells, and pro-inflammatory cytokines.[1] Brain inflammation in AD has been attributed to the potential immunogenicity of amyloid protein misfolding and aggregation, which is likely to occur 10–15 years before the clinical manifestation of AD.[2] However, whether brain inflammation occurs at the early, prodromal, or preclinical stage of AD remains unknown. Chronic brain inflammation presents in patients with multiple sclerosis (MS) or clinically isolated syndrome (CIS; a prodromal stage of MS), which can be assessed *in vivo* through measuring the cerebrospinal fluid (CSF) lymphocyte dynamics and pro-inflammatory cytokines.[3] Therefore, our goal was to determine whether brain inflammation occurs early in patients with amnestic mild cognitive impairment (aMCI), a preclinical stage of AD, and whether these changes can be revealed through measuring CSF immune components similar to those observed in patients with MS or CIS.

MATERIALS AND METHODS

All subjects and/or their study partners signed the written informed consent approved by the Institutional Review Boards of the UT Southwestern Medical Center and Texas Health Presbyterian Hospital of Dallas, in accordance with the Federal-wide Assurance on file with the Department of Health and Human Services (USA).

Subjects

Eleven aMCI patients aged 57–76 years participated in the study. The diagnosis of aMCI was based on the Petersen criteria,[4] as modified by the Alzheimer's Disease Neuroimaging Initiative project (http://adni-info.org) using a multidisciplinary diagnostic conference format. Clinical evaluations were performed at the UT Southwestern Medical Center Alzheimer's Disease Center, to exclude other conditions that may cause memory problems. The mean score of the Mini-Mental State Examination was 29.3 ± 0.8 and of the Wechsler Memory Scale Logical Memory for immediate and delayed recall were 11.0 ± 1.4 and 9.4 ± 0.9, respectively. All subjects had memory complaints and a global Clinical Dementia Rating score of 0.5 (memory box score $\geqslant 0.5$). Subjects were screened to exclude clinical histories of stroke, major medical and psychiatric disorders, unstable heart diseases, uncontrolled hypertension, diabetes mellitus, and chronic inflammatory diseases.

For comparison, 23 CIS/MS patients aged 20–69 years who participated in the study were evaluated at the UT Southwestern Medical Center Neurology Clinic or associated hospitals for possible MS diagnosis. The CIS patients in this cohort ($n = 17$) had experienced a single clinical demyelinating event, but did not meet the 2010 revised McDonald Criteria for MS at the time of sampling.[5] The MS patients in this cohort ($n = 6$) had also experienced at least one clinical demyelinating event, and all met the 2010 revised McDonald Criteria for MS at the time of sampling, including the presence of at least one brain lesion by magnetic resonance imaging.[5] Disease duration for the MS cohort was no longer than 2 years. Twelve of the CIS/MS patients presented with transverse myelitis, 7 of these patients presented with optic neuritis, and 4 of these patients presented with other symptoms. No CIS/MS patients were on therapy at the time of blood/CSF collection.

CSF Collection

CSF collection from aMCI and CIS/MS patients was performed at the Department of Neurology and Neurotherapeutics at the University of Texas Southwestern Medical Center by lumbar puncture. The volume of CSF collected ranged from 4–12 mL.

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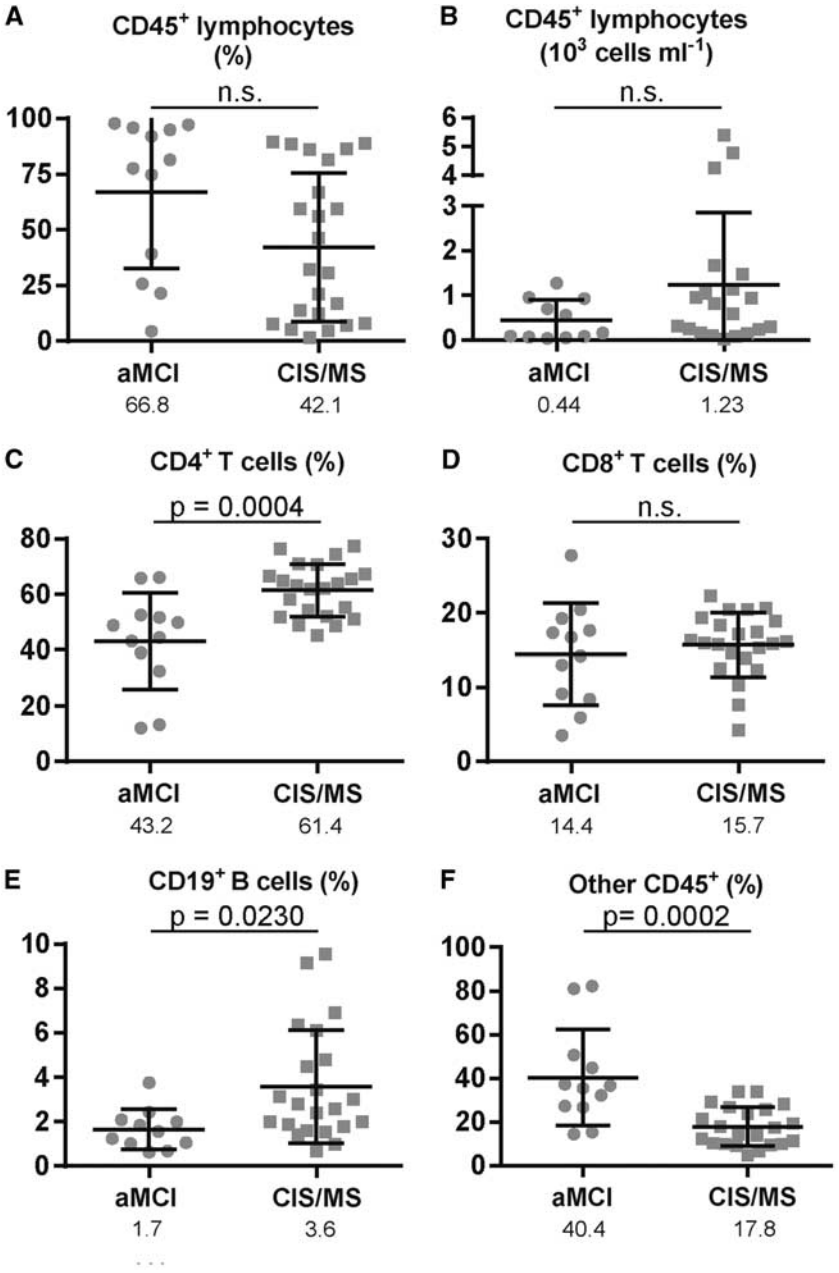

Figure 1. Lymphocyte dynamics in the cerebrospinal fluid (CSF) of amnestic mild cognitive impairment (aMCI) and clinically isolated syndrome/multiple sclerosis (CIS/MS) patients. CSF cells were pelleted and counted by a hemocytometer. (**A**) Percentage of $CD45^+$ lymphocytes obtained during the flow cytometry analysis. (**B**) Number of $CD45^+$ lymphocytes calculated from panel **A** and raw cell counts from the hemocytometer. (**C**–**F**) CSF cells were stained with markers to identify percentage of $CD4^+$ T cells (**C**), $CD8^+$ T cells (**D**), $CD19^+$ B cells (**E**), and other $CD45^+$ lymphocytes (**F**). *P*-values are indicated above each panel. The mean s.d. value for each scatter plot is indicated below the panels.

Flow Cytometry

Flow cytometry was performed as previously described.[6] Briefly, the CSF was centrifuged for 10 minutes at 394 *g* at 4 °C, the supernatant was collected, and banked at − 80 °C for enzyme-linked immunosorbent assay analysis. The remaining CSF cell pellet was gently resuspended in 200 μL ice-cold fluorescence-activated cell sorting buffer (1 × phosphate-buffered saline with 4% bovine serum albumin) and enumerated on a hemocytometer. The CSF pellet was stained with a multiparameter panel consisting of CD45 (APC-Cy7), CD4 (PE-Cy7), CD8 (APC), and CD19 (PerCP-Cy5.5 or Brilliant Violet 421) antibodies (BD Biosciences, San Jose, CA, USA). Cells were incubated on ice for 20 minutes in the dark, washed once in fluorescence-activated cell sorting buffer (453 *g*, 5 minutes at 4 °C), and resuspended in 100 μL fluorescence-activated cell sorting buffer. Cells were fixed with 2% paraformaldehyde for 20 minutes on ice and analyzed on a FACS Aria within 3 days (BD Biosciences). Supplementary Figure 1 provides a sample gating strategy for determining the percentage of $CD45^+$ lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, and $CD19^+$ B cells. 'Other' $CD45^+$ cells are defined as those $CD45^+$ cells that do not stain with CD4, CD8, or CD19.

Enzyme-Linked Immunosorbent Assay for Cytokine and Antibody Output Methods

Methods for enzyme-linked immunosorbent assay for cytokine and antibody output are available in the Supplementary Information.

Statistics

All statistical analyses comparing aMCI and CIS/MS cohorts were performed using Student's *t*-test.

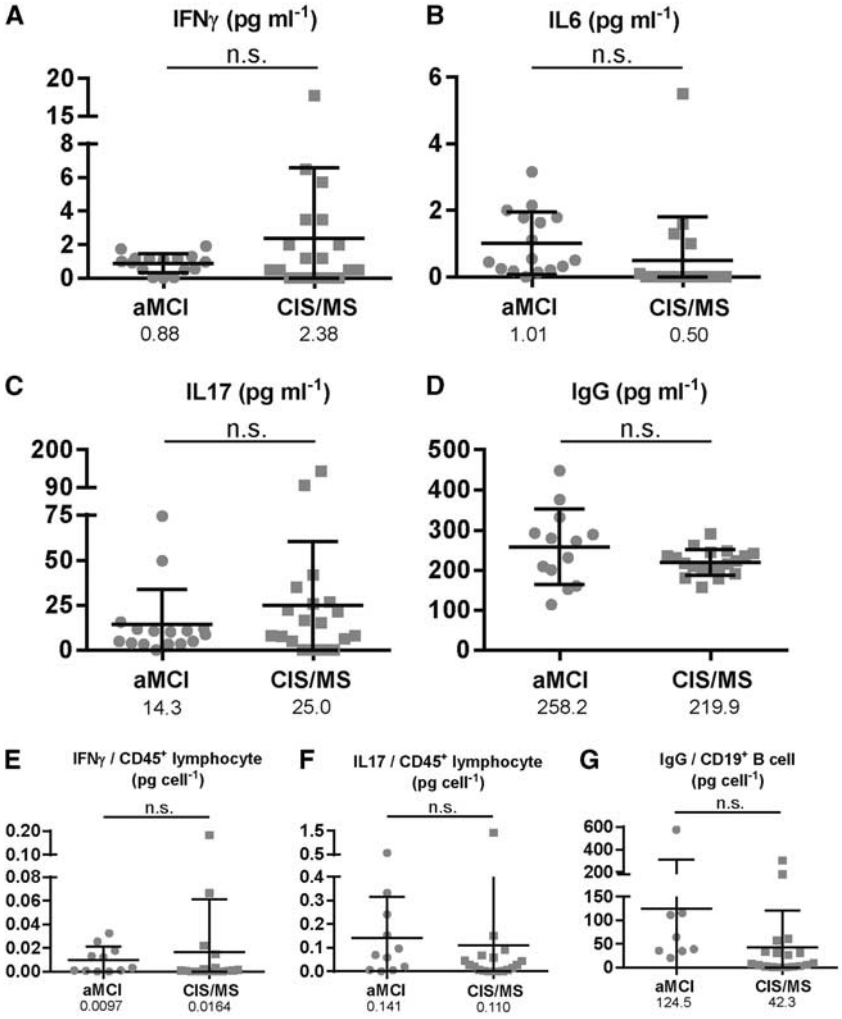

Figure 2. Cytokine and antibody profile in the cerebrospinal fluid (CSF) of amnestic mild cognitive impairment (aMCI) and clinically isolated syndrome/multiple sclerosis (CIS/MS) patients. CSF supernatants were evaluated for concentrations of interferon-γ (IFNγ; **A**), interleukin-6 (IL6; **B**), interleukin-17 (IL17; **C**), and IgG (**D**). The total amount of IFNγ by CD45$^+$ lymphocytes (**E**), IL17 by CD45$^+$ lymphocytes (**F**), and IgG by CD19$^+$ B cells (**G**) were calculated as described in Supplementary Methods. Means and s.d. are indicated in the scatter plots as the horizontal lines. All *P*-values are not significant (n.s.). The mean value for each scatter plot is indicated below the panels.

RESULTS

CSF cells stained with CD45 confirm their lymphocyte lineage (Figure 1A and 1B). aMCI patients averaged 66.8% CD45$^+$ lymphocytes in the CSF cell population, whereas CIS/MS patients averaged 42.1% CD45$^+$ lymphocytes. Interestingly, aMCI patients exhibited the similar wide range of CD45$^+$ distribution as CIS/MS patients, in both lymphocyte percent (Figure 1A) and absolute number (Figure 1B), suggesting the presence of different degrees of brain inflammation in aMCI patients. These observations were also confirmed when only those CIS/MS patients of similar age to the aMCI patients were considered (data not shown). Notably, more than half of both the patient groups exceeded the absolute number of CD45$^+$ lymphocytes typical of healthy donors[7] (660 lymphocytes/mL; data not graphed).

CD4$^+$ T cells are the predominant CSF lymphocyte population in CIS/MS patients (Figure 1C), in contrast to a reduced CD4$^+$ T-cell population in aMCI patients (CIS/MS 61.4%; aMCI 43.2%, $P = 0.0004$). However, age-matched CIS/MS patients had a similar CD4$^+$ T-cell frequency in the CSF compared with the aMCI cohort. The second largest adaptive immune cell population in the CSF of CIS/MS patients were CD8$^+$ T cells (15.7%, Figure 1D), was comparable in aMCI patients (14.4%), and the CD4:CD8 ratio also remained similar (compare 3.3 in aMCI with 3.4 in CIS/MS). CD19$^+$ B-cell representations were higher in the CIS/MS patients (3.6%; $P = 0.023$, Figure 1E) compared with the aMCI patients (1.7%). Although both CIS/MS and aMCI patients had a population of CD45$^+$ lymphocytes that were not T or B cells (thus defined as 'other' CD45$^+$ lymphocytes), aMCI patients had a statistically larger population of these 'other' CD45$^+$ lymphocytes (compare 17.8% in CIS/MS with 40.4% in aMCI; $P = 0.0002$, Figure 1F).

Cytokines in the CSF that reveal MS-induced neuroinflammation include interferon-γ , interleukin-6 (IL6), and interleukin-17 (IL17).[8] As expected, the average interferon-γ level in the CSF of CIS/MS patients is 2.38 pg/mL (Figure 2A). Interestingly, aMCI patients had a similar interferon-γ level (0.88 pg/mL, Figure 2A). IL6, IL17, and IgG levels in the CSF were also comparable in aMCI and CIS/MS patients (Figures 2B–2D). The output of interferon-γ, IL17, and IgG on a per-cell basis was the same between the aMCI and CIS/MS patients (Figures 2E–2G).

DISCUSSION

Patients with aMCI are at high risk to develop AD.[4] To our surprise, we found that $CD45^+$ lymphocytes and pro-inflammatory cytokines were present in the CSF of aMCI patients at levels similar to those found in untreated patients with CIS or MS, the prototypical neuroinflammatory disease. To our knowledge, there are no previous studies of lymphocyte immune pathologic assessment *in vivo* in the CSF of aMCI or AD patients. These data suggest that inflammatory processes may be important in the prodromal stages and development of AD.

Early studies had identified $CD4^+$ and $CD8^+$ T cells in the *post-mortem* brain tissue of AD patients,[9,10] suggesting a role for adaptive immunity in AD. Further evidence suggests a role for innate immunity in AD. Amyloid protein aggregates can trigger complement cascades and activate microglia and astrocytes.[1] Activated microglia and astrocytes also release pro-inflammatory cytokines, such as IL1, IL6, and tumor necrosis factor, at the sites of amyloid deposition.[1] Interestingly, changes similar to these in the brain parenchyma are clear indications of advancing immune pathologic assessment in other neurodegenerative diseases such as MS.[11]

Elevated CSF lymphocyte counts correlate closely with the number of gadolinium-enhancing lesions,[12] and elevated CSF antibody production is one of the established indicators of MS diagnosis.[5] Recently, flow cytometry techniques have been used to characterize the immune cell profiles in the CSF of patients with MS[13,14] and CIS, who are at risk to develop MS.[15] These studies demonstrate that T cells, a critical component of the adaptive immune system, dominate the CSF of MS and CIS patients. B cells are also present at greater numbers in the CSF of MS and CIS patients compared with that in control cohorts.[14] In combination, these CSF lymphocyte profile studies included a total of 335 MS patients, 120 CIS patients, and 95 control patients. None of the previous cohorts included patients with AD or aMCI.

It is also intriguing that CSF from the aMCI patients contained levels of the pro-inflammatory cytokines IL6 and IL17 that were comparable to untreated CIS/MS patients. This suggests that inflammatory processes occurring in the brain of aMCI patients involve both cellular and cytokine components. We did not observe any correlation of IL6 or IL17 levels in the CSF with T-cell frequency in either the aMCI or CIS/MS cohort. Nevertheless, as IL6 and IL17 are central to the development of pro-inflammatory T cells,[1,8] it will be of particular importance to determine whether T cells from aMCI patients respond functionally to pro-inflammatory stimuli in a manner similar to that of T cells from patients who have classic neuroinflammatory diseases of the central nervous system (CNS), such as MS.

The findings of this study reveal that brain inflammation is likely to occur at the preclinical stage of AD, which can be assessed *in vivo* in the CSF. Further study of CSF lymphocyte profiles, cytokine output and/or immunologic genetic signatures, and their relationships to amyloid β and τ-protein profiles may provide novel biomarkers to assess the extent of neuroinflammation in AD patients and those at higher risk to develop AD. Such findings may prompt the research community to re-evaluate the role of brain inflammation in AD pathophysiology and the development of therapeutic strategies designed to target improper immune responses or enhance beneficial immune mechanisms to counter AD pathologic study at its preclinical stage.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Journal of Cerebral Blood Flow & Metabolism website (http://www.nature.com/jcbfm)