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RESEARCH ARTICLE

Aβ-reactive T cell polyfunctionality response as a new biomarker for mild cognitive impairment

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Abstract

Introduction: Alzheimer's disease (AD) involves neuroinflammation and amyloid plaque deposition, yet the role of amyloid-reactive immune response in neurodegeneration remains unclear. We investigate amyloid-reactive T cell levels in the Epidemiology of Mild Cognitive Impairment Study in Taiwan (EMCIT) and Taiwan Precision Medicine Initiative of Cognitive Impairment and Dementia (TPMIC) cohorts.

Method: Using diverse amyloid peptide formulations, we established a polyfunctionality assay for five T cell functions and compared mild cognitive impairment (MCI) patients to control subjects in both cohorts.

Results: In both cohorts, MCI individuals exhibit higher amyloid-reactive T cell responses than controls. In the TPMIC cohort, CD4+ and CD8+ total response frequencies are notably elevated in MCI (CD4: 1.3%, CD8: 1.91%) versus controls (CD4: 0.15%, CD8: 0.28%; both $p < 0.001$). Amyloid-reactive T cell response outperforms plasma phosphorylated tau 181 (p-tau181) in discriminating MCI (area under the receiver operating characteristic curve CD4+: 0.97; CD8+: 0.96; p-tau181: 0.72; both $p < 0.001$).

Discussion: Amyloid-reactive T cell polyfunctional response distinguishes MCI from normal aging and could serve as a novel MCI biomarker.

KEYWORDS
amyloid, mild cognitive impairment, T cell response

Highlights

- Amyloid-reactive polyfunctional T cell responses can be detected in the peripheral circulation.
- Amyloid-reactive T cell response is significantly enhanced in individuals with mild cognitive impairment compared to age-matched, cognitively unimpaired individuals.
- The unique discriminative accuracy of amyloid-reactive T cell response is significantly higher than phosphorylated tau181 and is not a result of overall T cell hyperreactivity.
- Future studies are needed to determine the predictive role of amyloid-reactive T cell responses in disease progression and if the amyloid-reactive immune response could be a therapeutic target for the treatment of neurodegeneration.

1 | BACKGROUND

Alzheimer's disease (AD), the leading cause of dementia worldwide, is characterized by the accumulation of extracellular amyloid beta (Aβ) peptides within the brain.[1] With the global population aging, the World Health Organization estimates the total number of people with dementia is expected to increase rapidly from ≈ 26 million in 2005 to > 139 million by 2050, which presents a public health challenge of unprecedented magnitude.[2]

Converging evidence from both genetically at-risk cohorts and clinically normal older individuals suggests that the underlying pathology for AD, the most common form of dementia, begins years before the onset of clinical symptoms.[3] As a result, a state of early neurodegeneration, or mild cognitive impairment (MCI), is recognized as a phase in which individuals have cognitive symptoms of a mild nature but generally continue to function normally in the community. Individuals with MCI are at an alarming increased risk of progression to dementia, with a conversion rate of ≈ 10% to 15% per year.[4] Being an intermediate state between normal cognitive aging and dementia, MCI serves as a critical window for both preventive and therapeutic intervention to delay the occurrence of dementia during aging.[5]

Through years of research, the central role of Aβ in the pathogenesis of MCI and AD was established.[6] Numerous attempts have been made to investigate the prognostic role of deposition of brain amyloid and levels of Aβ in the cerebrospinal fluid (CSF) and peripheral blood.[7] The accumulation of amyloid β is evident in patients suffering from MCI[8] and drives the conversion from MCI to AD. Approximately 50% to 70% of patients with MCI showed significant cortical amyloid positron emission tomography (PET) retention.[9,10] Furthermore, in longitudinal studies assessing the effect of Aβ deposition on disease progression, MCI patients with high amyloid PET retention at baseline have a higher rate of progression to AD than those with low Aβ burden, with sensitivity ranging from 83.3% to 100% and specificity from 41.1% to 100%, respectively.[11] Thus, further understanding of the role of amyloid-associated pathogenetic mechanisms at the MCI stage is needed to design therapies aimed at reducing or eliminating Aβ from the brain.[12,13]

Neuroinflammation is an important aspect of neurodegeneration after the deposition of toxic amyloid plaques.[14,15] Deposition of amyloid plaques leads to the activation of microglial cells,[16] further inciting downstream proinflammatory responses[17] and recruiting T cells to the brain.[18] Studies have pointed out that aberrant secretion of proinflammatory cytokines by T cells contributes to the neuroinflammation[19] and modulation of the phagocytotic ability of microglia.[20] Dai and Shen showed that a small number of AD patients had an increased CD4+/CD8+ T cell ratio in the peripheral blood,[19] suggesting a disturbance of the immune system is involved in AD pathogenesis. Accumulating studies[21,22] indicate that diverse systemic phenotypic changes of immune cells are observed in AD and MCI. Recent data from > 360,000 participants from the UK Biobank[23] and China[24] further demonstrated peripheral immunity changes are associated with the risk of incident dementia. While these exciting studies point to the possibility of monitoring the peripheral immune system to detect cognitive decline, they lack the specificity of processes of neurodegeneration. These observations are thus subjective to numerous systemic and environmental factors that could impact an individual's overall immune homeostasis.

Protecting us against various pathogens and cancers, human T lymphocytes are characterized by their repertoire diversity and inter-individual variability.[25] Given the importance of Aβ in the pathogenesis of MCI and AD, we hypothesized that the T cell–specific (or, -reactive) immune response toward Aβ is involved in the disease course of neurodegeneration, and such changes can be captured in the periphery in MCI individuals. In this study, we tested the T cell polyfunctional response toward Aβ in MCI patients from two prospective cohort studies in Taiwan. Furthermore, we compared the amyloid-reactive T cell response to plasma phosphorylated tau 181 (p-tau181) on the accuracy of discriminating MCI from cognitively normal (CN) individuals.

2 | METHODS

2.1 | Human subjects

We included 69 patients with MCI and another 69 age-matched CN individuals from the Epidemiology of Mild Cognitive Impairment Study in Taiwan (EMCIT) study.[26] The participants reside in the communities of neighboring towns of the hospital in New Taipei City. Individuals with active cancer, recent hospitalization, or current infection were excluded. The study was approved by the Far Eastern Memorial Hospital (FEMH) institutional ethical committee (FEMH 105147-F). The study was carried out according to the ethical standard of FEMH, and written informed consent was obtained from all participants.

An additional 120 samples (41 MCI, 79 CN) were recruited from the Taiwan Precision Medicine Initiative of Cognitive Impairment and Dementia (TPMIC) study. The TPMIC study is an ongoing cohort initiated in 2021, with the ultimate enrollment of > 1000 elderly Taiwanese to build a precision medicine platform by integrating multiple modalities, including magnetic resonance imaging and PET neuroimaging, genetics, and immunoassay, to identify and integrate novel biomarkers linking progression from MCI to dementia. The participants were recruited from FEMH (FEMH 110065-F) and Cardinal Tien Hospital (CTH-110-2-1-014). Both institutions' ethical committees approved the TPMIC study.

For both cohorts, the diagnosis of MCI was confirmed by an expert panel comprising a neurologist, a psychiatrist, and a clinical psychologist, based on National Institute on Aging–Alzheimer's Association criteria.[27]

2.2 | Sample processing and peripheral blood mononuclear cells isolation

For all samples, the blood was sampled when cognitive function tests were performed. On the day of blood sampling, peripheral blood mononuclear cells (PBMCs) were immediately isolated by Ficoll-Paque PLUS gradient centrifugation following the manufacturer's protocol (GE Healthcare). PBMCs were frozen in liquid nitrogen for long-term preservation.

2.3 | Full-length (1-42) and peptide pool of Aβ protein

The full-length Aβ protein (Aβ1-42, A21G, Flemish variant)[28] amino acid sequence is DAEFRHDSGYEVHHQKLVFFGEDVGSNKGAIIGLMVGGVVIA. The full-length wild-type peptide sequence (both from JPT Peptide Technologies) is DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA.

Using protein-spanning mixtures of overlapping peptides is highly efficient for stimulating T lymphocytes for diagnostic applications. A peptide pool composed of 10 sequences created based on 15mer amino acid length with sequential three amino acid shifts of the A21G full-length Aβ protein (Aβ1-42) was produced by Kelowna Internal Scientific Inc. The sequences are the following: DAEFRHDSGYEVHHQ (Aβ1-15), FRHDSGYEVHHQKLV (Aβ4-18), DSGYEVHHQKLVFFG (Aβ7-21), YEVHHQKLVFFGEDV (Aβ10-24), HHQKLVFFGEDVGSN (Aβ13-27), KLVFFGEDVGSNKGA (Aβ16-30), FFGEDVGSNKGAIIG (Aβ19-33), EDVGSNKGAIIGLMV (Aβ22-36), GSNKGAIIGLMVGGV (Aβ25-39), and KGAIIGLMVGGVVIA (Aβ28-42). For tests on EMCIT samples, the AβA21G peptide pool and A21G full-length peptide were used for T cell stimulation. For tests on TPMIC cohort samples, Aβ1-42 full-length wild-type peptide was also used.

RESEARCH IN CONTEXT

1. **Systemic review**: The authors reviewed current literature on Alzheimer's disease (AD) and mild cognitive impairment (MCI) biomarkers based on peripheral immune response using diverse assays available in PubMed. Several studies have pointed to the general involvement of the immune system and T cells in AD, but the role of amyloid-reactive T cells has not been explored in MCI, nor has the head-to-head comparison to existing blood-based biomarkers been made.
2. **Interpretation**: Our results demonstrate the feasibility of detecting peripheral T cell response toward amyloid peptides using the polyfunctionality assay. Such response is significantly elevated in patients with MCI. The discriminative accuracy of amyloid T cell response is also superior to plasma phosphorylated tau 181.
3. **Future directions**: Our study proposes to use amyloid-reactive T cell response as a novel biomarker for MCI. The ongoing Taiwan Precision Medicine Initiative of Cognitive Impairment and Dementia longitudinal follow-up results will extend our findings. In addition, amyloid-reactive T cell response also holds the potential to become a modifiable therapeutic target.

2.4 | T cell stimulation and quantification of amyloid-specific T cell response

T cell stimulation and polyfunctionality analyses were performed as described in our previous studies.[29,30] The full-length Abeta protein (WT and A21G) and the amyloid peptide pool were used for PBMC cell stimulation along with co-stimulation (anti-CD28/CD49d), anti-CD107a, Golgistop (monensin), and Golgiplug (brefeldin A) for 6 hours at 37°C. Afterward, cells were stained with anti-CD3, anti-CD8, anti-CD4, and Live/Dead cell viability assay (Invitrogen) for 20 minutes before fixation with Cytofix/Cytoperm buffer (BD Biosciences). Cells were fixed, washed, and stained with anti-CD40L, anti-IL-2, anti-TNFα, and anti-IFNγ. Results were acquired using a multicolor flow cytometer (Beckman Coulter Cytoflex) at the FEMH Core Lab. Flow

cytometry results were analyzed using FlowJo (Tree Star). After gated on live CD3+ cells, CD4+ and CD8+ cells were analyzed separately for cytokine expression in response to each stimulation (Figure S1 in supporting information). Frequency of reactive cells expressing each effector function among CD4+ or CD8+ T cells was determined. A combinatorial gating strategy based on the gates of each effector function was further applied to determine co-expression statistics using the FlowJo Boolean gate platform. The total amyloid-specific T cell response frequency was derived by summing the percentages of amyloid-reactive cells expressing at least one effector function among total CD8+ or CD4+ T cells (% CD8+ or % CD4+).[29]

2.5 | Polyfunctionality assay controls

For all PBMC stimulation conditions, a mock control (without peptide stimulation) was used as a background control. In addition, the chemicals PMA/ionomycin were used as positive controls to stimulate all T cells as a reference of total T cell reactivity. For TPMIC samples, an additional unrelated cytomegalovirus (CMV) antigen (pp65 peptide pool, JPT Peptide Technologies) was also tested.

2.6 | p-tau181 measurement

p-tau181 was measured at Veritas laboratory (Taipei, Taiwan). The Simoa Human pTau181 Advantage V2 assay was used according to the manufacturer's protocol using the Simoa HD-X analyzer.

2.7 | Statistical analyses

Comparing T cell responses between groups was investigated using the Student *t* test. The area under the receiver operating characteristic curve (AUROC) was used to examine the discriminative performance of these effector functions as potential biomarkers. AUROCs and their confidence intervals (CIs) were calculated based on 2000 bootstrap samples using the non-parametric method (rocreg command in STATA). Due to age difference between MCI and CN adults in the TPMIC sample, age-adjusted AUROCs were additionally calculated by regressing continuous age in the model. The equality of AUROCs between p-tau181 and T cell effector functions was tested using roccomp command in STATA. All statistical analyses were conducted in STATA 17 (StataCorp LLC).

3 | RESULTS

3.1 | Study population

Detailed demographic information on EMCIT and TPMIC study participants is listed, respectively, in Table S1 and Table S2 in supporting information.

3.2 | T cell reactivity against Aβ peptide demonstrates a distinct polyfunctionality profile

We and others have used multicolor flow cytometry to monitor human virus antigen-specific T cell polyfunctionality.[31–33] An intracellular cytokine detection polyfunctionality assay detects multiple cytokine T cells generated in response to specific antigen challenges simultaneously on a single-cell basis and is used to monitor infectious disease activity,[34] vaccine efficacy,[35] and immune protection.[36] However, polyfunctional response toward amyloid peptide has never been reported. Figure S1 shows the representative staining of amyloid-reactive polyfunctional T cell response. The combinatorial polyfunctional contribution of each effector function is shown in Figure S2 in supporting information. CD4+ amyloid-reactive CD4+ T cells are tumor necrosis factor alpha (TNF-α) producers, while some also produce interferon gamma (IFN-γ) and interleukin (IL)-2. CD8+ amyloid-reactive T cells are mostly IFN-γ and CD107a producers, which is compatible with the higher cytotoxic potential of CD8+ T cells (Figure S2). Both CD4+ and CD8+ subsets produce variable levels of CD40L, a co-stimulation molecule indicating recent T cell activation. Compared to our previous analyses on human immunodeficiency virus–reactive[32] or CMV-reactive T cells,[29] which exhibited a high percentage of co-expression of TNF-α and IFN-γ, amyloid-reactive polyfunctional response patterns are distinct in that a large percentage of CD4+ T cells produce TNF-α alone and a large percentage of CD8+ T cells produce IFN-γ alone. Thus, the polyfunctional response pattern of amyloid-reactive T cells is unique and different from other antigenic T cell responses against viruses.

3.3 | Enhanced Aβ-specific T cell response in MCI individuals of the EMCIT cohort

Next, we compared the magnitude of T cell responses against Aβ full-length peptide A21G and the amyloid A21G peptide pool between MCI participants with CN individuals from the EMCIT cohort (Table 1). In CD4+ cells, MCI individuals exhibited statistically significantly higher amyloid-specific responses in almost all individual effector functions (CD107a, IFN-γ, IL-2, and TNF-α, except CD40L) and the combined total response (TR, which integrated the response magnitude from all five functions tested). Amyloid-reactive CD4+ T cells TR was significantly increased in MCI patients compared to CN individuals (mean [standard deviation (SD)]: 0.79% [0.73] vs. 0.27% [0.35], $p < 0.001$). For CD8+ T cells, the differences were milder, but amyloid-reactive CD8+ T cells TR was also increased in MCI individuals compared to CN individuals (mean [SD]: 0.67% [0.80] vs. 0.59% [0.4], $p = 0.026$). The comparisons failed to reach statistical significance for most single CD8+ functions, except for IL-2. Notably, there were no differences in the control stimulation condition (PMA/ionomycin, which activates all T cells) between MCI and control participants (data not shown). This indicates that the overall functionality of all the T cells in a given individual was not different between MCI and CN, and analyzing the overall functionality without antigen specificity will not be able to distinguish MCI from CN. p-tau181 is an established biomarker for AD

TABLE 1 Comparisons of amyloid-specific T cell response between cognitively normal elderly and MCI individuals in the EMCIT cohort.

	CD4+ T cell			CD8+ T cell		
Biomarker mean (SD)	Normal ($n = 69$)	MCI ($n = 69$)	p value	Normal (n = 69)	MCI (n = 69)	p value
Amyloid pool TR	0.27 (0.35)	0.79 (0.73)	< 0.001**	0.40 (0.59)	0.67 (0.80)	0.026*
CD40L	0.12 (0.20)	0.088 (0.14)	0.28	0.19 (0.43)	0.25 (0.42)	0.47
CD107a	0.05 (0.095)	0.25 (0.32)	< 0.001**	0.091 (0.18)	0.15 (0.28)	0.13
IFN-γ	0.051 (0.096)	0.17(0.19)	< 0.001**	0.094 (0.14)	0.18 (0.24)	0.015*
IL-2	0.034 (0.056)	0.12 (0.14)	< 0.001**	0.036 (0.09)	0.094 (0.20)	0.033*
TNF-α	0.088 (0.22)	0.29 (0.46)	0.001*	0.048 (0.11)	0.088 (0.25)	0.24
Amyloid full TR	0.51 (0.61)	0.93 (0.73)	< 0.001**	0.54 (0.74)	0.77 (1.11)	0.14
CD40L	0.1 (0.11)	0.14 (0.25)	0.29	0.19 (0.38)	0.25 (0.56)	0.46
CD107a	0.043 (0.074)	0.21 (0.27)	< 0.001**	0.12 (0.28)	0.19 (0.42)	0.27
IFN-γ	0.043 (0.071)	0.18 (0.18)	< 0.001**	0.19 (0.27)	0.14 (0.16)	0.27
IL-2	0.061 (0.093)	0.18 (0.21)	< 0.001**	0.054 (0.086)	0.17 (0.53)	0.074
TNF-α	0.36 (0.58)	0.45 (0.60)	0.37	0.11 (0.19)	0.14 (0.19)	0.52

Note: TR, total T cell response frequency of CD4+ or CD8+ T cells. Values are shown as mean (SD).
Abbreviations: EMCIT, Epidemiology of Mild Cognitive Impairment Study in Taiwan; IFN-γ, interferon gamma; IL-2, interleukin 2; MCI, mild cognitive impairment; TNF-α, tumor necrosis factor alpha; SD, standard deviation; TR, total response.
*p value < 0.05.
**p value < 0.001.

that is elevated in the initial stage of MCI even when only subtle changes in brain pathology are detected.[37,38] In the EMCIT cohort, we found that MCI individuals also demonstrated higher levels of plasma p-tau181 compared to CN subjects, although the differences did not reach statistical significance (mean [SD]: 3.22 [2.26] pg/mL vs. 2.66 [1.68] pg/mL, $p = 0.1$).

The AUROC was used to examine the discriminative performance of these effector functions (CD40L, CD107a, IFN-γ, IL-2, TNF-α, and TR) as potential biomarkers between MCI and CN subjects (Figure 1). The AUROC for the total CD4+ T cell responses to full-length amyloid peptide was 0.83 (CI: 0.76–0.9; Figure 1A). The highest AUROC was 0.86 for CD107a. The AUROC for the amyloid peptide pool responding to CD4+ T cell TR response was 0.72 (CI: 0.64–0.81), with the highest AUROC being 0.84 for IFN-γ (Figure 1C). In both stimulation conditions, CD4+ T cell TR frequency performed better than plasma p-tau181 (AUROC: 0.59 [0.5–0.69]; $p = 0.01$ compared to CD4+ full-length stimulation; $p = 0.051$ compared to CD4+ peptide pool stimulation; Table S3 in supporting information) in predicting MCI versus CN. The discriminative ability of CD8+ T cell responses was less satisfactory and similar to p-tau181 (Figure 1B,D). In summary, CD4+ amyloid-specific responses exhibit good performance in separating MCI from CN when either full-length amyloid peptide–based stimulation or peptide pool–based stimulation was used. CD8+ T cell responses and p-tau181 were both less accurate.

3.4 | Enhanced Aβ-specific CD4+ and CD8+ T cell response in MCI individuals from the TPMIC cohort

To validate the above findings based on participants from the EMCIT cohort, we selected an additional 41 MCI individuals and 79 CN individuals from the TPMIC cohort (for demographic data, see Table S2). In addition to the A21G full-length amyloid peptide and A21G amyloid peptide pool used in EMCIT cohort samples, another peptide sequence, the Aβ1-42 wild-type amyloid full-length peptide, was also included for T cell stimulation (Table 2) for TPMIC samples. In contrast to results from the EMCIT cohort, MCI individuals in the TPMIC cohort demonstrated significantly higher levels of plasma p-tau181 compared to CN subjects (mean [SD]: 4.04 [2.47] pg/mL vs. 2.01 [0.90] pg/mL, $p < 0.001$).

The magnitude of each effector function elicited from full-length wild type Aβ1-42 amyloid and full-length A21G amyloid peptide were similar (Table 2). Consistent with the findings from EMCIT cohort samples, both CD4+ and CD8+ T cell response parameters were significantly increased in MCI individuals compared to CN. However, the differences were greater for TPMIC samples. In all three amyloid stimulation conditions (and among both CD4+ and CD8+ T cell populations), the frequency of amyloid-reactive T cells was at least four to six times higher in MCI than in CN individuals (Table 2; Figures S3, S4 in supporting information).

The AUROC analyses of amyloid-reactive T cell responses and plasma p-tau181 in TPMIC MCI patients versus CN subjects are shown in Figure 2 and Table S4 in supporting information. Among all the T cell response parameters, successful discrimination between MCI and CN was achieved in TPMIC samples, especially with the total T cell response frequency (TR, age-adjusted AUROC > 0.9 in all stimulation conditions), which incorporated the response from all five effector functions tested. Along with TR, all single T cell function parameters exhibited age-adjusted AUROC > 0.8. p-tau181 exhibited an age-adjusted AUROC of 0.72 (CI: 0.59–0.84). All the amyloid-reactive TR parameters (both CD4+ and CD8+ T cells) exhibited statistically

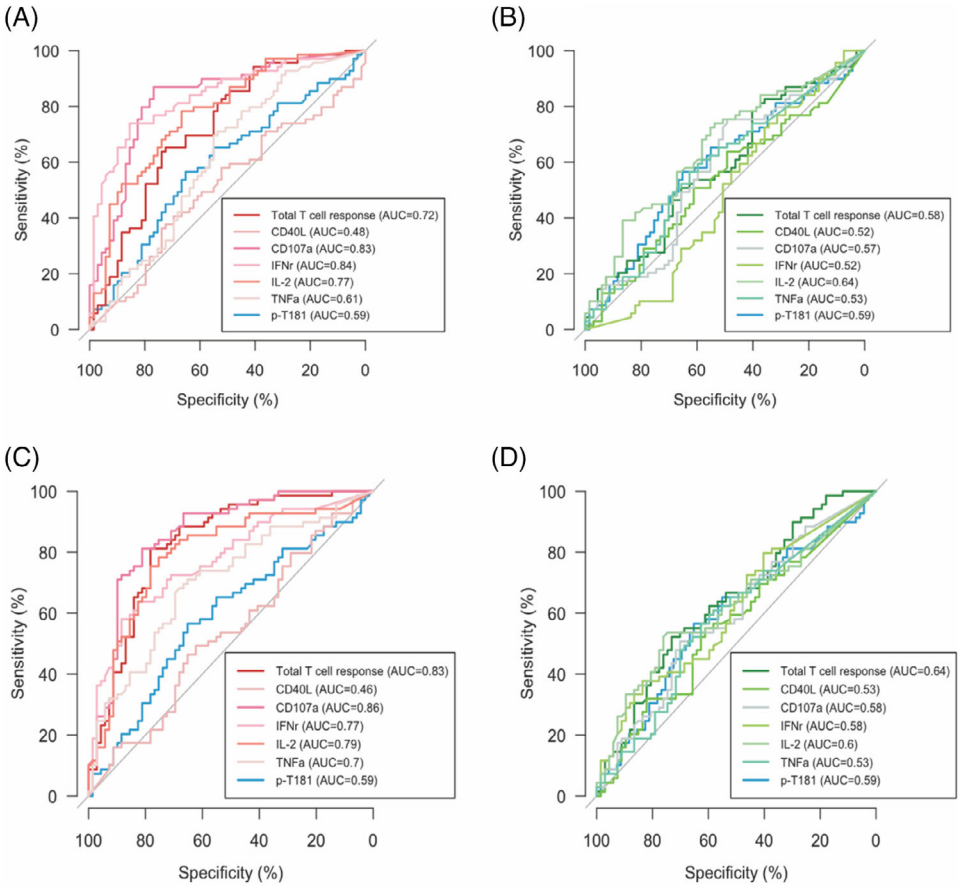

FIGURE 1 AUROC curve analysis of T cell responses and plasma p-tau181 in MCI patients ($n = 69$) versus CN subjects ($n = 69$) of the EMCIT study. A, CD4+ T cell amyloid peptide pool response. B, CD8+ T cell amyloid peptide pool response. C, CD4+ T cell amyloid full-length A21G peptide response. D, CD8+ T cell amyloid full-length A21G peptide response. AUROC, area under the receiver operating characteristic; CN, cognitively normal; EMCIT, Epidemiology of Mild Cognitive Impairment Study in Taiwan; MCI, mild cognitive impairment; p-tau181, phosphorylated tau 181.

significantly superior discriminative accuracy compared to p-tau181 to distinguish MCI from CN.

We additionally tested the discriminative accuracy of CMV-specific T cell response (using the dominant pp65 protein peptide pool) and PMA/ionomycin stimulation (Table S5 in supporting information). In both experimental conditions (CMV or PMA/ionomycin-induced), polyfunctionality response AUROC was much inferior to either p-tau181 or amyloid-reactive responses (Table S5).

Several previous reports suggest that peripheral T cell differentiation status is associated with AD.[22,39,40] We thus further checked the T cell subset differentiation status in TPMIC cohort samples (Table S6 in supporting information). We found that T cell differentiation status was similar between MCI and CN subjects. Based on these findings, non–antigen-specific, general T cell immune status differences did not distinguish MCI individuals from CN subjects. As a result, peripheral blood-derived amyloid T cell polyfunctionality response uniquely differentiates individuals with MCI from normal elderly.

Because results from the TPMIC cohort showed superior distinguishability by amyloid T cell polyfunctionality to separate MCI from CN subjects compared to results from the EMCIT cohort, we then performed a sensitivity analysis to understand the potential factors involved in the difference. MCI individuals from the EMCIT cohort had milder disease; thus, separating them from CN individuals is likely more challenging. The average Mini-Mental State Examination (MMSE) score of MCI individuals in the EMCIT cohort was 24.9, while the average MMSE score of MCI individuals in the TPMIC cohort was 22. We thus excluded individuals with MMSE < 17 in the TPMIC cohort and analyzed the distinguishability by amyloid T responses. As shown in Table S7 in supporting information, the modified TPMIC cohort has a higher MMSE score compared to the EMCIT cohort. However, amyloid T responses still demonstrated significant distinguishability between MCI and CN individuals in the modified TPMIC cohort (Table S8 in supporting information).

4 | DISCUSSION

Identification of novel blood-based biomarkers for preclinical dementia and MCI is an urgent need in the field of dementia research that will provide crucial therapeutic intervention opportunities.[41,42] Our

TABLE 2 Comparisons of amyloid-specific T cell response between cognitively normal elderly and MCI individuals in the TPMIC cohort.

	CD4+ T cell			CD8+ T cell		
Biomarker mean (SD)	Normal ($n = 79$)	MCI ($n = 41$)	p value	Normal ($n = 79$)	MCI ($n = 41$)	p value
Amyloid pool TR	0.15 (0.31)	1.30 (0.86)	< 0.001**	0.28 (0.34)	1.91 (1.28)	< 0.001**
CD40L	0.04 (0.07)	0.28 (0.22)	< 0.001**	0.12 (0.23)	0.81 (1.11)	< 0.001**
CD107a	0.03 (0.06)	0.42 (0.50)	< 0.001**	0.14 (0.26)	0.68 (0.59)	< 0.001**
IFN-γ	0.02 (0.02)	0.20 (0.17)	< 0.001**	0.12 (0.23)	0.35 (0.24)	< 0.001**
IL-2	0.04 (0.06)	0.23 (0.23)	< 0.001**	0.07 (0.13)	0.51 (0.62)	< 0.001**
TNF-α	0.10 (0.30)	0.42 (0.33)	< 0.001**	0.07 (0.16)	0.23 (0.25)	< 0.001**
Amyloid full TR A21G	0.10 (0.16)	1.11 (0.60)	< 0.001**	0.30 (0.69)	1.97 (1.18)	< 0.001**
CD40L	0.04 (0.09)	0.31 (0.22)	< 0.001**	0.13 (0.24)	0.73 (0.91)	< 0.001**
CD107a	0.03 (0.08)	0.34 (0.31)	< 0.001**	0.10 (0.23)	0.67 (0.73)	< 0.001**
IFN-γ	0.03 (0.06)	0.20 (0.20)	< 0.001**	0.13 (0.64)	0.52 (0.39)	< 0.001**
IL-2	0.02 (0.04)	0.23 (0.20)	< 0.001**	0.06 (0.09)	0.41 (0.32)	< 0.001**
TNF-α	0.04 (0.09)	0.25 (0.20)	< 0.001**	0.03 (0.06)	0.20 (0.19)	< 0.001**
Amyloid full TR WT	0.15 (0.26)	1.24 (0.69)	< 0.001**	0.33 (0.97)	2.17 (1.42)	< 0.001**
CD40L	0.08 (0.23)	0.28 (0.22)	< 0.001**	0.12 (0.22)	0.84 (0.98)	< 0.001**
CD107a	0.04 (0.11)	0.36 (0.27)	< 0.001**	0.09 (0.15)	0.87 (1.24)	< 0.001**
IFN-γ	0.03 (0.07)	0.24 (0.24)	< 0.001**	0.18 (0.93)	0.54 (0.57)	0.024*
IL-2	0.04 (0.07)	0.29 (0.28)	< 0.001**	0.07 (0.12)	0.46 (0.49)	< 0.001**
TNF-α	0.06 (0.13)	0.32 (0.29)	< 0.001**	0.03 (0.06)	0.27 (0.25)	< 0.001**

Note: TR: total T cell response frequency of CD4+ or CD8+ T cells. Values are shown as mean (SD).
Abbreviations: IFN-γ, interferon gamma; IL-2, interleukin 2; MCI, mild cognitive impairment; SD, standard deviation; TPMIC, Taiwan Precision Medicine Initiative of Cognitive Impairment and Dementia; TNF-α, tumor necrosis factor alpha; TR, total response; WT, wild type.
*p value < 0.05.
**p value < 0.001.

results indicate that T cell responses toward full-length amyloid peptide and amyloid peptide pools are both significantly enhanced in MCI individuals. This observation is novel and unique because other antigen-induced T cell responses were not simultaneously increased in MCI individuals. In addition, amyloid-specific T cell responses separate MCI individuals from CN individuals more accurately than plasma p-tau181. Because enhanced immune cell activation and neuroinflammation are critical processes involved in the development and propagation of MCI and AD,[14,15] our findings implicate that circulatory amyloid-specific T cell responses can potentially be used to monitor disease activity and predict the prognosis of patients with cognitive decline.

The differential findings between the EMCIT and TPMIC cohorts, especially regarding the CD8+ T cell responses, are worthy of discussion. In the EMCIT cohort, both CD8+ T cells and p-tau181 failed to distinguish MCI from CN individuals. However, they both could satisfactorily distinguish MCI from CN in the TPMIC cohort. The sensitivity test we performed in Tables S7 and S8 showed that the severity of cognitive impairment is not responsible for the difference. In contrast to the EMCIT cohort, significant differences between MCI and CN individuals in age, cardiovascular disease, history of smoking, and apolipoprotein E ε4 carrier percentage were observed in the TPMIC cohort. As a result, it is possible that more MCI individuals in the TPMIC cohort could be in the AD spectrum than MCI individuals in the EMCIT cohort.

To the best of our knowledge, this current study is the first report investigating amyloid-specific T cell response in MCI. Several previous publications had investigated amyloid-reactive T cell response in the AD stage using older technologies and yielded conflicting results. An earlier study[43] found that T cell reactivity toward Aβ peptides was enhanced during aging and trended even higher in patients with AD based on proliferation assays and cytokine measurements in the supernatants after T cells were stimulated with amyloid peptides for 48 hours. In that study, there was no distinction between CD4+ and CD8+ T cells. Another study[44] investigated the peripheral blood lymphocyte subtypes of AD patients and identified that CD8+ T_{EMRA} cells were increased in AD and also clonally expanded in AD patients' CSF. A recent report by Dhanwani et al.[45] suggested T cell responses toward several essential neuronal proteins involved in neurodegeneration, including Aβ, amyloid precursor protein, tau, and alpha-synuclein, were similar between AD patients and age-matched controls. This current study shows that CD4+ and CD8+ T cell amyloid-specific responses were both increased in MCI individuals. Importantly, because the unrelated control response (CMV) was not able to distinguish MCI versus control, the enhanced amyloid-reactive T cell response is not a result of overall T cell hyperreactivity. Nevertheless, besides patient population

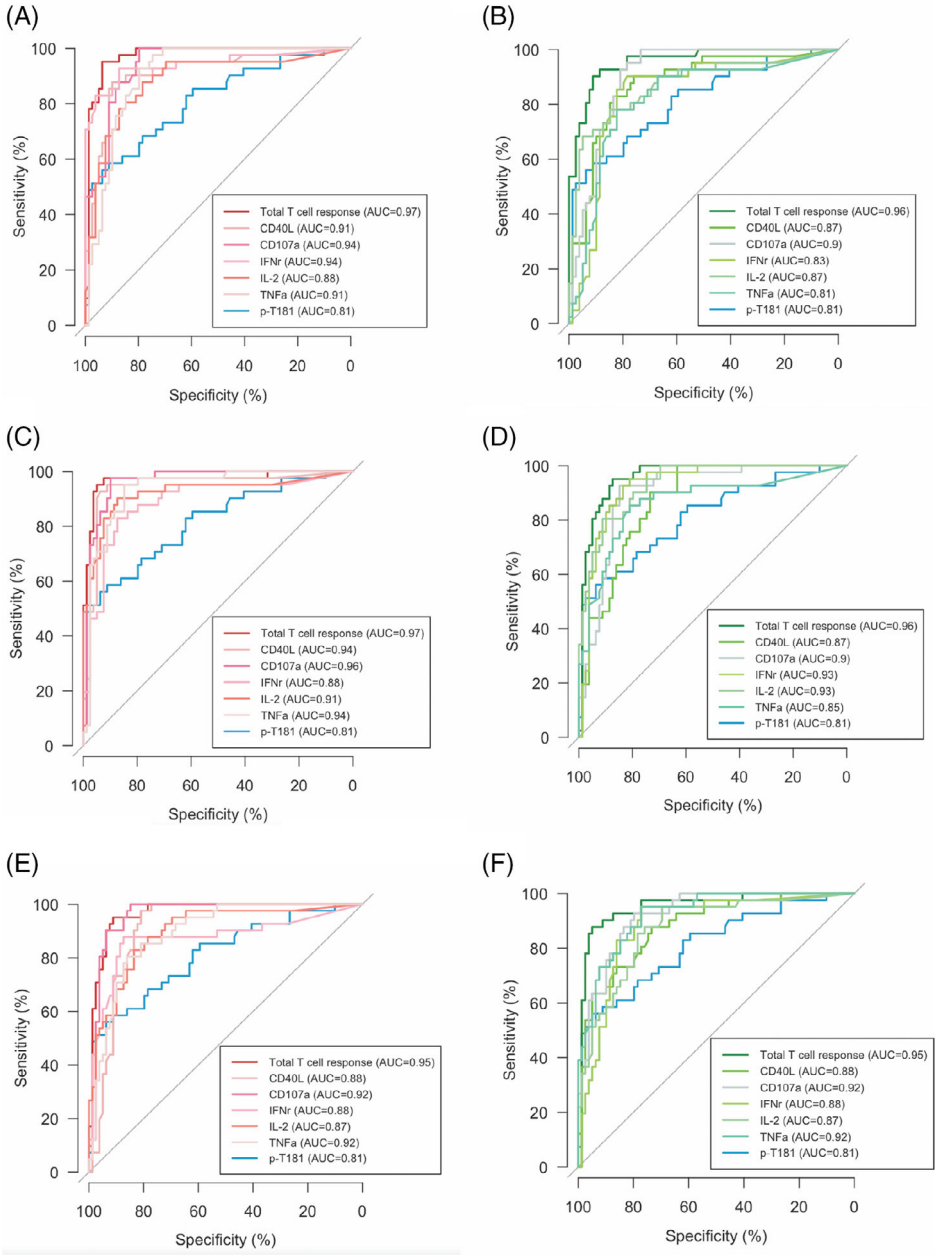

FIGURE 2 FIGURE 2 AUROC curve analysis of T cell responses and plasma p-tau181 in MCI patients ($n = 41$) versus CN subjects ($n = 79$) of the TPMIC study. A, CD4+ T cell amyloid peptide pool response. B, CD8+ T cell amyloid peptide pool response. C, CD4+ T cell amyloid full-length A21G peptide response. D, CD8+ T cell amyloid full-length A21G peptide response. E, CD4+ T cell amyloid full-length wild-type peptide response. F, CD8+ T cell amyloid full-length wild-type peptide response. AUROC, area under the receiver operating characteristic; CN, cognitively normal; MCI, mild cognitive impairment; p-tau181, phosphorylated tau 181; TPMIC, Taiwan Precision Medicine Initiative of Cognitive Impairment and Dementia.

difference (AD vs. MCI), significant differences exist in the methodology adopted for T cell assays compared to the current study. Geginat et al. used a 2-week long triple-color IFN-γ, IL-10, and IL-5 Fluorospot assay, which critically depends on the continuous in vitro proliferation and survival of antigen-specific T cells, which is subjective to significant biases from non–antigen-specific T cells responding to cytokines in the culture medium.[46] On the other hand, our study adopted a 6 hour–only polyfunctionality assay, which makes it easier to acquire consistent responses than cellular proliferation over days. Because reactivity toward amyloid peptides is limited to a very small population of all T cells, our multi-marker approach increased the absolute value of measurements to the range > 0.1% and demonstrated the superior ability to distinguish MCI from CN individuals compared to p-tau181.

The role of the adaptive immune response in the pathogenesis of MCI and AD remains an evolving field of research. Enhanced T cell response against Aβ might be detrimental as it could enhance neuroinflammation and facilitate neurodegeneration. In a recent animal study,[47] CD4+, amyloid-specific effector T cell clones, when

transferred into a susceptible transgenic mouse model (APP/PS1) of AD, accelerated memory impairment and increased brain amyloid burden. It has also been shown that IFN-γ production by amyloid-specific Th1 cells, but not Th2 or IL-17–producing cells, increased microglial activation and plaque burden in APP/PS1 mice.[48] Future interventions to deplete amyloid-reactive T cells or increase amyloid-reactive regulatory cells might provide therapeutic benefits.

The primary strengths of our study include the large sample size and detailed characterization of amyloid-reactive T cell response parameters. Nevertheless, our study has several important limitations. First, because this is a cross-section analysis, the ability of amyloid-specific T cell responses to predict the progression of cognitive decline still needs to be investigated. Second, study participants with available amyloid PET scan results are few. Without amyloid PET scan results, it is unclear how many MCI individuals in this study are within the AD spectrum. Thus, the generalizability of amyloid T response and p-tau181 in this study population is limited. The role of amyloid-specific T cell responses in amyloid-positive versus amyloid-negative MCI patients should be explored in the future. These questions will be investigated in the TPMIC cohort as the participants enter the follow-up phase and PET scan results become available. Finally, CyTOF (cytometry by time of flight) technology, which is capable of more cellular markers and broader analyses, could provide additional insights into the distinction between MCI and normal individuals.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests related to this study. The authors have declared that there is no conflict of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

The study was approved by the FEMH institutional ethical committee (FEMH 105147-F). The study was carried out according to the ethical standards of FEMH. All human subjects provided informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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