Amyloid beta-specific T cell response is enhanced in individuals with mild cognitive impairment

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Abstract

Background:

Neuroinflammation and deposition of amyloid plaques are key features of patients with Alzheimer’s dementia or mild cognitive impairment (MCI), but little is known about the role of amyloid-reactive immune response in cognitive decline. Using an amyloid b-specific T cell polyfunctionality assay, we tested samples from the Epidemiology of Mild Cognitive Impairment in Taiwan study (EMCIT) and the Taiwan Precision Medicine Initiative of Cognitive Impairment and dementia (TPMIC) study.

Results:

Both cohorts showed enhanced amyloid-reactive T-cell responses in individuals with MCI. In the EMCIT cohort, the individual’s amyloid peptide pool-reactive CD4+ and CD8+ total response frequencies were significantly larger in MCI patients (n=69; CD4+: 0.79%; CD8+: 0.67%) than in control individuals (n=69; CD4+: 0.27%; CD8: 0.4%; both p < 0.05). Notably, CD4+ T cell total response discriminated MCI versus control (AUROC, 0.72) with significantly higher accuracy than p-Tau181 (AUROC: 0.59, p < 0.01). In the TPMIC cohort, both amyloid peptide pool-reactive CD4+ and CD8+ total response frequencies were also higher in MCI individuals (n=41; CD4: 1.3%, CD8: 1.91%) than in control (n=79, CD4: 0.15%, CD8: 0.28%; both p < 0.001). Amyloid peptide pool-reactive total CD4+ and CD8+ T cell response frequencies outperformed p-Tau181 in their discriminative accuracy of MCI (CD4+ AUROC, 0.97; CD8+ AUROC, 0.96; p-Tau181 AUROC, 0.72; both p < 0.001). Other amyloid peptide formulations similarly induced an increase of T cell response in MCI individuals and demonstrated superior discriminative accuracy than p-Tau181.

Conclusion:

Our study indicates that T cell-specific, amyloid-associated T cell response increases in individuals with MCI. T cell response against amyloid is a novel biomarker of mild cognitive impairment. Further studies are needed to investigate the potential role of amyloid-T cell response as a risk factor for future cognitive decline.

Background

Alzheimer’s disease (AD), the leading cause of dementia worldwide, is characterized by the accumulation of extracellular β-amyloid peptides (amyloid β, Aβ) within the brain. With the global population aging, the total number of people with dementia is expected to increase from around 26 million in 2005 to over 80 million by 2040, which presents a public health challenge of unprecedented magnitude (1).

Converging evidence from both genetically at-risk cohorts and clinically normal older individuals suggests that the underlying pathology for Alzheimer’s disease (AD), the most common form of dementia, begins years before the onset of clinical symptoms (2). As a result, a state of early neurodegeneration, or mild cognitive impairment (MCI), has come to be recognized as a phase whereby
individuals may have cognitive symptoms of a mild nature but generally continue to function normally in the community (3). Current data indicate that individuals with MCI are at an alarming increased risk of progression to dementia, with a conversion rate of approximately 10–15% per year (4, 5).

Through years of research, the central role of amyloid β in the pathogenesis of MCI and AD was established. Numerous attempts have been made to investigate the prognostic role of deposition of brain amyloid (6) and levels of amyloid β in the cerebral spinal 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 50–70% of patients with MCI showed significant cortical amyloid PET retention (9, 10). Furthermore, in longitudinal studies assessing the effect of amyloid β 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 83.3–100% and specificity from 41.1–100%, respectively (11). Thus, detection of Aβ pathology at the pre-symptomatic stages of AD may help assess the potential effects of Aβ deposition on cognition and neurodegeneration and, equally importantly, identify individuals that may be most likely to benefit from therapies aimed at reducing or eliminating Aβ from the brain.

Nevertheless, besides the deposition of toxic amyloid plaques, there is another important aspect of neurodegeneration, which is the neuroinflammation (12, 13). The formation of amyloid plaques leads to the activation of microglial cells (14), further inciting downstream proinflammatory responses (15) and recruiting T cells to the brain (16). Studies have also pointed out that aberrant secretion of proinflammatory cytokines by T cells contributes to the neuroinflammation (17) and modulation of the phagocytotic ability of microglia (18). Given the importance of amyloid β in the pathogenesis of AD and MCI, we hypothesized that the T cell-specific immune response toward amyloid β is involved in the disease course of neurodegeneration. In this study, we tested the ex vivo T cell polyfunctional response toward amyloid using either full-length or peptide pool amyloid β in MCI patients from two prospective cohort studies and compared with plasma p-Tau181 on the accuracy to discriminate MCI from cognitively normal individuals.

Results

Study population

Detailed demographic information on EMCIT and TPMIC study participants is listed in Supplementary Table 1 and Supplementary Table 2, respectively.

Polyfunctional T cell response against amyloid β peptide

The representative staining of polyfunctional T cell response against the amyloid A21G peptide pool is shown in Supplementary Fig. 1. In virtually all samples, CD4 + amyloid-reactive CD4 + T cells are TNFα producers, while some also produce IFNγ and IL-2. CD8 + amyloid-reactive T cells are mostly IFNγ and
CD107a producers, which is compatible with the higher cytotoxic potential of CD8+ T cells. Both CD4+ and CD8+ subsets produce CD40L, a costimulation molecule indicating recent T cell activation.

**Enhanced amyloid β-specific T cell response in MCI individuals of the EMCIT cohort**

First, we compared the magnitude of T cell responses against amyloid β full-length peptide A21G and the amyloid A21G peptide pool between MCI participants with cognitively normal individuals (CN) 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, IFNγ, IL-2, and TNFα, except CD40L) and the combined total response (TR, which integrated the response magnitude from all five functions tested). Amyloid-reactive CD4+ T cells were significantly increased in MCI patients than in CN individuals (mean [SD]: 0.79% [0.73] versus 0.27% [0.35], p < 0.001). For CD8+ T cells, the differences were milder, but amyloid-reactive CD8+ T cells were also increased in MCI individuals than in CN individuals (mean [SD]: 0.67% [0.80] versus 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), indicating that the overall functionality of total T cells was not different between MCI and CN. MCI individuals also demonstrated higher levels of plasma p-Tau181 compared with CN subjects, although the differences did not reach statistical significance (mean [SD]: 3.22 [2.26] pg/ml versus 2.66 [1.68] pg/ml, p = 0.1).
Table 1
Comparisons of Amyloid-specific T cell response between cognitively normal elderly and MCI individuals in the EMCIT cohort

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>CD4 + T cell</th>
<th>CD8 + T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 69)</td>
<td>MCI (n = 69)</td>
</tr>
<tr>
<td>Amyloid pool TR</td>
<td>0.27 (0.35)</td>
<td>0.79 (0.73)</td>
</tr>
<tr>
<td>CD40L</td>
<td>0.12 (0.20)</td>
<td>0.088 (0.14)</td>
</tr>
<tr>
<td>CD107a</td>
<td>0.05 (0.095)</td>
<td>0.25 (0.32)</td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.051 (0.096)</td>
<td>0.17 (0.19)</td>
</tr>
<tr>
<td>IL2</td>
<td>0.034 (0.056)</td>
<td>0.12 (0.14)</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.088 (0.22)</td>
<td>0.29 (0.46)</td>
</tr>
<tr>
<td>Amyloid full TR</td>
<td>0.51 (0.61)</td>
<td>0.93 (0.73)</td>
</tr>
<tr>
<td>CD40L</td>
<td>0.1 (0.11)</td>
<td>0.14 (0.25)</td>
</tr>
<tr>
<td>CD107a</td>
<td>0.043 (0.074)</td>
<td>0.21 (0.27)</td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.043 (0.071)</td>
<td>0.18 (0.18)</td>
</tr>
<tr>
<td>IL2</td>
<td>0.061 (0.093)</td>
<td>0.18 (0.21)</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.36 (0.58)</td>
<td>0.45 (0.60)</td>
</tr>
</tbody>
</table>

TR: total T cell response frequency of CD4+ or CD8+ T cells. Values are shown as mean (SD). **: p value < 0.001. *: p value < 0.05.

The area under the receiver operating characteristic curve (AUROC) was used to examine the discriminative performance of these effector functions (CD40L, CD107a, IFNγ, IL-2, and TNFα) as potential biomarkers between MCI and CN subjects (Fig. 1). The AUROC for the total CD4 + T cell responses to full-length amyloid peptide was 0.83 (CI: 0.76–0.9) (Fig. 1A). The highest AUROC was 0.86 for CD107α. The AUROC for the amyloid peptide pool responding to CD4 + T cell response was 0.72 (CI: 0.64–0.81), with the highest AUROC being 0.84 for IFNγ (Fig. 1C). In both stimulation conditions, CD4 + T cell total response (TR) frequency performed better than plasma p-Tau181 (AUROC: 0.59 [0.5–0.69]; p =
0.01 when compared with CD4 + full-length stimulation, p = 0.051 when compared with CD4 + peptide pool stimulation, Supplementary Table 3) in predicting MCI versus CN. The discriminative ability of CD8 + T cell responses was less satisfactory and similar to p-Tau181 (Fig. 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.

Enhanced amyloid β-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 additional 41 MCI individuals and 79 CN individuals from the TPMIC cohort (for demographic data, see Supplementary Table 2). In addition to the A21G full-length amyloid peptide and A21G amyloid peptide pool used in EMCIT cohort samples, the Aβ1-42 wild-type amyloid full-length peptide was also included for T cell stimulation (Table 2) for TPMIC samples. The magnitude of each effector function elicited from full-length wild type Aβ1-42 amyloid and full-length A21G amyloid peptide were similar. 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 fold increases were higher 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 4–6 times higher in MCI than in CN individuals (Table 2).
Table 2
T cell response against amyloid β peptide pool response is enhanced in MCI individuals from the TPMIC cohort

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>CD4 + T cell</th>
<th>CD8 + T cell</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 79)</td>
<td>MCI (n = 41)</td>
<td>P value</td>
<td>Normal (n = 79)</td>
<td>MCI (n = 41)</td>
<td>P value</td>
</tr>
<tr>
<td>Amyloid pool TR</td>
<td>0.15 (0.31)</td>
<td>1.30 (0.86)</td>
<td>&lt; 0.001**</td>
<td>0.28 (0.34)</td>
<td>1.91 (1.28)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>CD40L</td>
<td>0.04 (0.07)</td>
<td>0.28 (0.22)</td>
<td>&lt; 0.001**</td>
<td>0.12 (0.23)</td>
<td>0.81 (1.11)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>CD107a</td>
<td>0.03 (0.06)</td>
<td>0.42 (0.50)</td>
<td>&lt; 0.001**</td>
<td>0.14 (0.26)</td>
<td>0.68 (0.59)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.02 (0.02)</td>
<td>0.20 (0.17)</td>
<td>&lt; 0.001**</td>
<td>0.12 (0.23)</td>
<td>0.35 (0.24)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>IL2</td>
<td>0.04 (0.06)</td>
<td>0.23 (0.23)</td>
<td>&lt; 0.001**</td>
<td>0.07 (0.13)</td>
<td>0.51 (0.62)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.10 (0.30)</td>
<td>0.42 (0.33)</td>
<td>&lt; 0.001**</td>
<td>0.07 (0.16)</td>
<td>0.23 (0.25)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Amyloid full TR</td>
<td>0.10 (0.16)</td>
<td>1.11 (0.60)</td>
<td>&lt; 0.001**</td>
<td>0.30 (0.69)</td>
<td>1.97 (1.18)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>A21G</td>
<td>0.10 (0.16)</td>
<td>1.11 (0.60)</td>
<td>&lt; 0.001**</td>
<td>0.30 (0.69)</td>
<td>1.97 (1.18)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>CD40L</td>
<td>0.04 (0.09)</td>
<td>0.31 (0.22)</td>
<td>&lt; 0.001**</td>
<td>0.13 (0.24)</td>
<td>0.73 (0.91)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>CD107a</td>
<td>0.03 (0.08)</td>
<td>0.34 (0.31)</td>
<td>&lt; 0.001**</td>
<td>0.10 (0.23)</td>
<td>0.67 (0.73)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.03 (0.06)</td>
<td>0.20 (0.20)</td>
<td>&lt; 0.001**</td>
<td>0.13 (0.64)</td>
<td>0.52 (0.39)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>IL2</td>
<td>0.02 (0.04)</td>
<td>0.23 (0.20)</td>
<td>&lt; 0.001**</td>
<td>0.06 (0.09)</td>
<td>0.41 (0.32)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.04 (0.09)</td>
<td>0.25 (0.20)</td>
<td>&lt; 0.001**</td>
<td>0.03 (0.06)</td>
<td>0.20 (0.19)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Amyloid full TR</td>
<td>0.15 (0.26)</td>
<td>1.24 (0.69)</td>
<td>&lt; 0.001**</td>
<td>0.33 (0.97)</td>
<td>2.17 (1.42)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>WT</td>
<td>0.15 (0.26)</td>
<td>1.24 (0.69)</td>
<td>&lt; 0.001**</td>
<td>0.33 (0.97)</td>
<td>2.17 (1.42)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>CD40L</td>
<td>0.08 (0.23)</td>
<td>0.28 (0.22)</td>
<td>&lt; 0.001**</td>
<td>0.12 (0.22)</td>
<td>0.84 (0.98)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>CD107a</td>
<td>0.04 (0.11)</td>
<td>0.36 (0.27)</td>
<td>&lt; 0.001**</td>
<td>0.09 (0.15)</td>
<td>0.87 (1.24)</td>
<td>&lt; 0.001**</td>
</tr>
</tbody>
</table>

TR: total T cell response frequency of CD4 + or CD8 + T cells. WT: wild-type. Values are shown as mean (SD). **: p value < 0.001. *: p value < 0.05.
### Table 1: Biomarker Mean (SD) for CD4+ and CD8+ T cell responses

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>CD4+ T cell</th>
<th>CD8+ T cell</th>
<th>P value</th>
</tr>
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<tr>
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<td>Normal (n = 79)</td>
<td>MCI (n = 41)</td>
<td></td>
</tr>
<tr>
<td>IFNγ</td>
<td>0.03 (0.07)</td>
<td>0.24 (0.24)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>IL2</td>
<td>0.04 (0.07)</td>
<td>0.29 (0.28)</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.06 (0.13)</td>
<td>0.32 (0.29)</td>
<td>&lt; 0.001**</td>
</tr>
</tbody>
</table>

TR: total T cell response frequency of CD4+ or CD8+ T cells. WT: wild-type. Values are shown as mean (SD). **: p value < 0.001. *: p value < 0.05.

The area under the receiver operating characteristic curve (AUROC) analyses of T cell responses and plasma p-Tau181 in MCI patients versus CN subjects were shown in Fig. 2 and Supplementary Table 4. 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. Phospho-Tau181 exhibited an age-adjusted AUROC of 0.72 (CI: 0.59–0.84). All the TR parameters (both CD4+ and CD8+ T cells) exhibited statistically significant superior discriminative accuracy than p-Tau181 to distinguish MCI from CN.

### Discussion

Identification of novel blood-based biomarkers for preclinical dementia and MCI is an urgent need in the field of dementia research, which will provide crucial therapeutic intervention opportunities (23, 24). Our results indicated that peripheral T cell responses toward full-length amyloid peptide and amyloid peptide pool are significantly enhanced in MCI individuals. In addition, amyloid-specific T cell response separates MCI individuals from CN individuals more accurately than plasma p-Tau181. Since enhanced immune cell activation (25) and neuroinflammation are critical processes involved in the development and propagation of MCI and AD (12, 13), our findings implicate that circulatory amyloid-specific T cell responses can potentially be used to monitor disease activity and potentially predict the prognosis of patients with cognitive decline.

One might be curious about the differential findings between the EMCIT and TPMIC cohorts, especially regarding the CD8+ T cell responses. In the EMCIT cohort, CD8+ T cells failed to distinguish MCI from CN individuals. Similarly, pTau-181 also failed to distinguish MCI from CN individuals in the EMCIT cohort but could satisfactorily distinguish MCI from CN in the TPMIC cohort. MCI individuals from the EMCIT cohort may have milder disease and, thus, are more challenging to separate them from cognitively...
normal individuals. The average MMSE score of MCI individuals in the EMCIT cohort was 24.9, while the average MMSE score of MIC individuals in the TPMIC cohort was 20.1.

While amyloid-related immune responses have been investigated in previous publications and yielded conflicting results, this report is the first study showing enhanced amyloid-specific T cell functionality against amyloid peptide stimulations in MCI individuals and, to our knowledge, contains the most study participants. Dai et al. showed that a small number of AD patients had an increased CD4+/CD8+ T cell ratio in the peripheral blood (17), suggesting a disturbance of the immune system is involved in AD pathogenesis. An earlier study (26) indicated that T cell reactivity toward amyloid \( \beta \) peptides was enhanced during aging and trended toward 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 recent report (27) investigated the peripheral blood lymphocyte subtypes of AD patients and identified that CD8 + \( T_{EMRA} \) cells were increased in AD. These CD8 + \( T_{EMRA} \) cells also clonally expanded in AD patients' cerebral spinal fluid. This current study shows that CD4+ and CD8+ T cell amyloid-specific responses were both increased in MCI individuals.

Our results, while encouraging, contrast with a recent by Dhanwani et al. (28), which suggested T cell responses toward several essential neuronal proteins involved in neurodegeneration, including amyloid \( \beta \), amyloid precursor protein, tau, and alpha-synuclein, were similar between AD patients and age-matched controls. Nevertheless, there exist significant differences in the methodology adopted for T cell assays. Notably, Dhanwani et al. used a two-week long triple-color IFN\( \gamma \), IL-10, and IL-5 Fluorospot assay, which critically depends on the continuous \textit{in vitro} proliferation and survival of antigen-specific T cells. In addition, such a method is subjective to significant biases from non-antigen-specific T cells responding to cytokines in the culture medium (29). On the other hand, our study adopted a 6-hour-only polyfunctionality assay to visualize amyloid-reactive T responses and measurements of intracellular cytokine effector response by multicolor flow cytometry, which is easier to acquire consistent responses compared to cellular proliferation over days. In addition, 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 above 0.1% and demonstrated the superior ability to distinguish MCI from cognitively normal individuals than p-Tau 181.

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 amyloid \( \beta \) might be detrimental as it could enhance neuroinflammation and facilitate neurodegeneration. In a recent animal study (30), 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-\( \gamma \) production by amyloid \( \beta \)-specific Th1 Cells, but not Th2 or IL-17-producing cells, increased microglial activation and plaque burden in APP/PS1 mice (31).
Our study has several limitations. First, since this is a cross-section study, 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. In the future, the association between PET scan results and amyloid-specific T cell responses should be explored. Finally, besides the accumulation of extracellular amyloid β plaques, other key neuropathological features seen in the post-mortem brains of AD patients are generalized cortical atrophy, neuronal and synaptic loss, and intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau (34). Whether additional imaging and blood-based biomarkers can be used in combination with our amyloid-specific polyfunctionality assay for risk stratification of MCI patients requires further investigation.

**Conclusions**

Amyloid-specific polyfunctional and individual T cell responses are significantly enhanced in individuals with MCI patients compared to age-matched individuals. The discriminative accuracy of amyloid-reactive T cell response is significantly higher than p-Tau181. Future studies are needed to determine the potential role of amyloid-reactive T cell responses in predicting future cognitive decline.

**Methods**

*Human Subjects*

We included 69 patients with MCI and another 69 age-matched cognitively normal (CN) individuals from the Epidemiology of Mild Cognitive Impairment study in Taiwan (EMCIT) study (19). All human subjects were recruited from Far Eastern Memorial Hospital. 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 FEMH’s institutional ethical committee (FEMH 105147-F), and written informed consent was acquired 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 more than one thousand elderly Taiwanese to build a precision medicine platform by integrating multiple modalities, including MRI 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 comprised of a neurologist, a psychiatrist, and a clinical psychologist, based on NIA-AA criteria (3).

*Sample processing and PBMCs 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.

**Full-length (1-42) and peptide pool of amyloid β protein**

The full-length amyloid β protein (Ab1-42, A21G, Flemish variant) (20) amino acid sequence is DAEFRHDSGYEVHHQKLKVFFGEDVGSNKGAILLMVGGVVIA. The full-length wild-type peptide sequence (both from JPT Peptide Technologies) is DAEFRHDSGYEVHHQKLVFKAEDVGSNKGAILLMVGGVVIA. Using protein-spanning mixtures of overlapping peptides is highly efficient for stimulating T-lymphocytes for diagnostic applications. We additionally created the overlapping 15mer peptides derived from the full-length amyloid β protein A21G variant.

Peptide pool composed of 10 sequences created based on 15mer amino acid length with sequential three amino acid shifts of the A21G full-length amyloid β protein (Ab1-42) was produced by Kelowna Internal Scientific Inc. The sequences are as the following: DAEFRHDSGYEVHHQ (Ab1-15), FRHDSGYEVHHQKLV (Ab4-18), DSGYEVHHQKLFF (Ab7-21), YEVHHQKLVFFEDV (Ab10-24), HHQKLVFFEDVG (Ab13-27), KLFFEDVGSK (Ab16-30), FFFGSDVGSKG (Ab19-33), EDVGSKGAI (Ab22-36), GSKGAI (Ab25-39) and KGAIIGLMVGGVIA (Ab28-42). For tests on EMCIT samples, the AbA21G peptide pool and A21G full-length peptide were used for T cell stimulation. For tests on TPMIC cohort samples, Ab1-42 full-length wild-type peptide was also used. The chemicals PMA/ionomycin were used as positive controls.

**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 (21, 22). The full-length amyloid b protein, as well as the amyloid peptide pool, were used for PBMC cell stimulation (1mg/ml per peptide) along with costimulation (anti-CD28/CD49d), anti-CD107a, Golgistop (monensin) and Golgiplug (brefeldin A) for six 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 Cytotix/Cytopherm 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 Far Eastern Memorial Hospital 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 (Supplementary Table 1). 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+) (21).
**Phospho-Tau 181 Measurement**

Phospho-Tau 181 (pTau181) 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.

**Statistical Analyses**

Comparing T cell responses between groups was investigated using the Student’s t-test. The area under the receiver operating characteristic curve (ROC) was used to examine the discriminative performance of these effector functions as potential biomarkers. Areas under the curve (AUROCs) and their confidence intervals (CI) were calculated based on 2000 bootstrap samples using the non-parametric method (rocreg command in STATA). Due to age difference between MCI and cognitively normal adults in the TPMIC sample, age-adjusted AUROCs were additionally calculated by regressing continuous age in the model. The equality of AUROCs between p-tau 181 and T-cell effector functions was tested using roccomp command in STATA. All statistical analyses were conducted in STATA 17 (College Station, TX: StataCorp LLC).

**Declarations**

The authors have declared that there is no conflict of interest.

**Data availability**

Original experimental data is available upon request.

**Author contributions**

Yen-Ling Chiu, Yi-Fang Chuang designed the study and provided the funding source; Sui-Hing Yan, Yi-Chien Liu and Yi-Fang Chuang recruited study participants; Yen-Ling Chiu, Yi-Fang Chuang, Yi-Chien Liu, Yang-Teng Fan, Chiung-Fang Chang, Ruo-Wei Hung, and TienYu Owen Yang performed tests on subjects, processed the data, performed experiments, and analyzed the results; Yen-Ling Chiu and Yi-Fang Chuang wrote the manuscript and submitted the manuscript.

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**Competing Interests**

The authors declare no conflict of interest related to this study.

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References


**Figures**
Figure 1

Area under the receiver operating characteristic (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.
Area under the receiver operating characteristic (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.
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