

Increased Senescent CD4⁺ T Cell Subsets In Alzheimer’s Disease Patients Significantly Correlate With AD Biomarkers And Memory Dysfunction



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ABSTRACT

Background: Senescent and exhausted T cells accumulate with aging and various diseases and likely contribute to inflammation associated with Alzheimer’s disease (AD). The control of naïve/memory T cell populations in AD versus normal aging is largely undefined. We hypothesize that AD individuals have dysregulated proportions of memory T cells subsets compared to age-matched controls and that these differences influence disease pathogenesis.

Methods: Using flow cytometric analyses, the frequencies of naïve/memory T cell phenotypes were assessed in peripheral blood mononuclear cells from individuals with AD (n=18) and age-matched controls with no cognitive impairment (NCI; n=4). Plasma inflammatory and AD markers, including cytokines, chemokines, amyloid beta (A β) peptides, and tau protein modifications, were measured using MesoScale Discovery assays. Memory and cognitive functions were calculated using the Tablet-based Cognitive Assessment Tool (TabCAT) digital platform.

Results: Within memory CD4⁺ T cells subsets, “Terminal effector memory re-expressing CD45RA” or TEMRA were significantly increased in AD participants compared to NCI. Furthermore, senescent CD4⁺ T cells (either CD57⁺ or CD28^{neg}) were significantly higher (p < 0.05) in AD, while exhausted CD4⁺ T cells (KLRG1⁺) trended higher (p < 0.1 > 0.05). Conversely, no significant differences in any of the CD8⁺ T cell subsets were observed. In the plasma, significant increases in inflammatory and disease markers were also detected in the AD group, including osteopontin, neurofilament light chain (NfL), GFAP, and phosphorylated forms of tau protein (ptau T217 and T181). The levels of CD4⁺ TEMRA positively correlated with measures of progressing AD disease, namely NfL levels and the ratios of ptau T217 or T181 over total tau protein. Strikingly, the frequencies of CD4⁺ TEMRA and CD28^{neg} cells negatively correlated with the TabCAT measures of multimodal associative memory, particularly delayed memory. The correlations between exhausted T cell levels (KLRG1⁺) and phosphorylated tau or inflammatory markers also trended as positive (p < 0.1).

Conclusion: The current findings suggest that senescent CD4⁺ cells are actively associated with neurodegenerative processes and cognitive decline in AD individuals and may serve as potential blood-based indicators for disease monitoring and severity.

Cohort Demographics

Table 1	NCI (Non-Cognitively Impaired)	AD (Alzheimer’s Disease)	p value
Sample size (n)	4	18	n.a.
Age yrs (mean $\pm$ SD)	66.5 $\pm$ 7.6	72.8 $\pm$ 4.9	0.197
MoCA Scores (mean $\pm$ SD)	27.8 $\pm$ 1.5	19.7 $\pm$ 5.6	<0.0001
Female	3 (75%)	9 (50%)	n.a.
Male	1 (25)	9 (50%)	
Anti-amyloid therapy n (%)	n.a.	14 (77.8%)	n.a.

INTRODUCTION

Senescent T cells are known to accumulate in normal aging and to contribute to an increase in background levels of inflammation that is termed *inflammaging*. PMID:38502582

- Increased senescent cells are predominantly of the CD8⁺ T cell subset.
- Senescent CD4⁺ T cells do not accumulate in normal aging.

Situations that lead to increases in senescent CD4⁺ T cells:

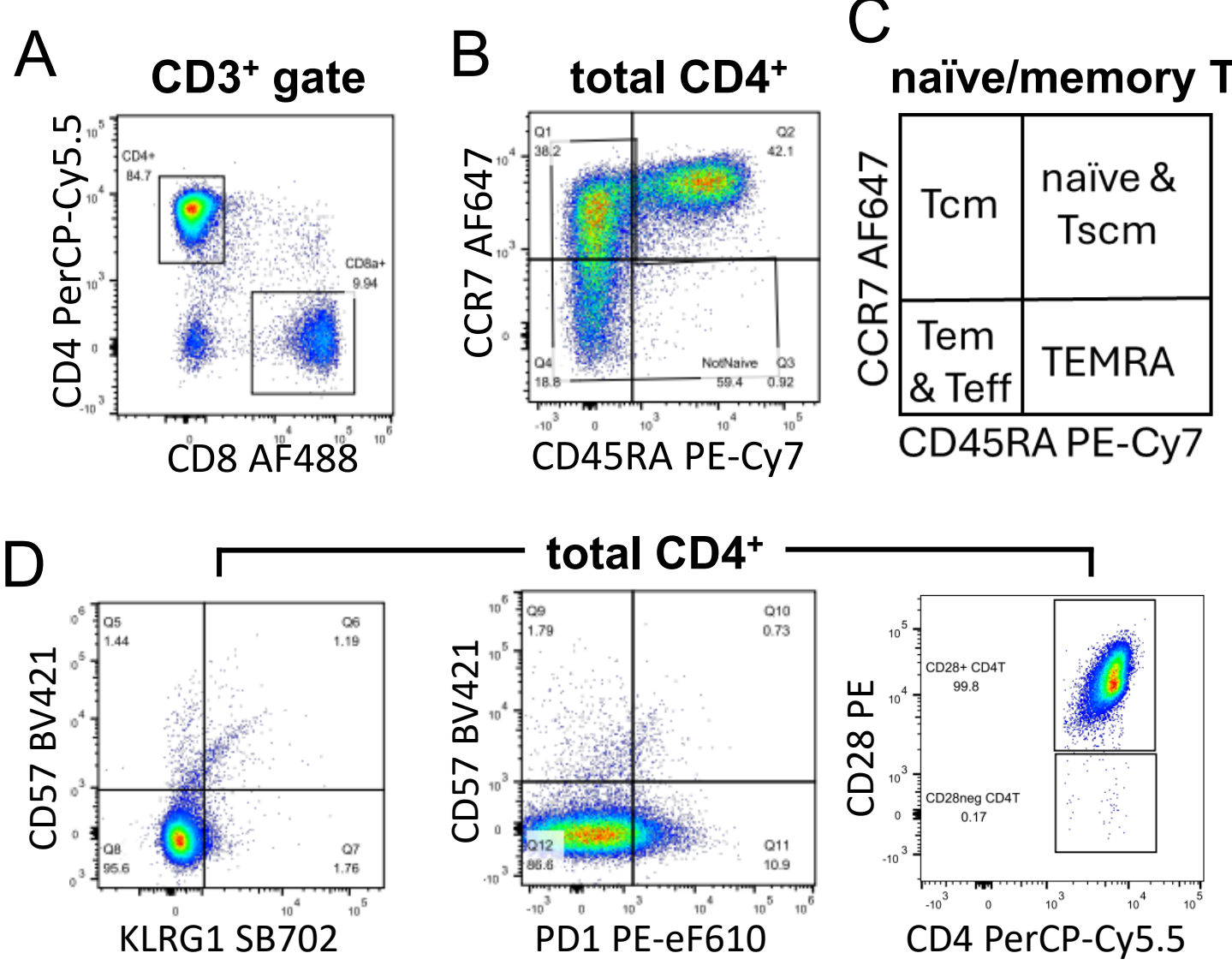
- Leishmania-infected lesions PMID: 39497830
- Advanced lupus disease PMID: 38752316
- Rheumatoid arthritic joints PMID: 24313359
- SARS-CoV-2 infection PMID: 39298288

Increased senescent CD4⁺ T cells are associated with increased severity of various disease states. Therefore, we hypothesized that **senescent T cell phenotypes will be increased in our AD cohort compared to age-matched controls.**

Methods

- PBMC T cells from AD (n=18) or age-matched NCI individuals (n=4) were assessed by 14-color flow cytometry for naïve/memory and exhausted/senescent phenotypes in CD4⁺ and CD8⁺ subsets.
- Total RNA was isolated from PBMC and the relative expression of senescence-associated and inflammatory genes in AD compared to NCI individuals was determined by qPCR.
- Plasma levels of inflammatory and AD-associated markers were determined by ELISA or MSD assays.
- Memory and cognitive functions were assessed using TabCAT-Brain Health Assessment tools.

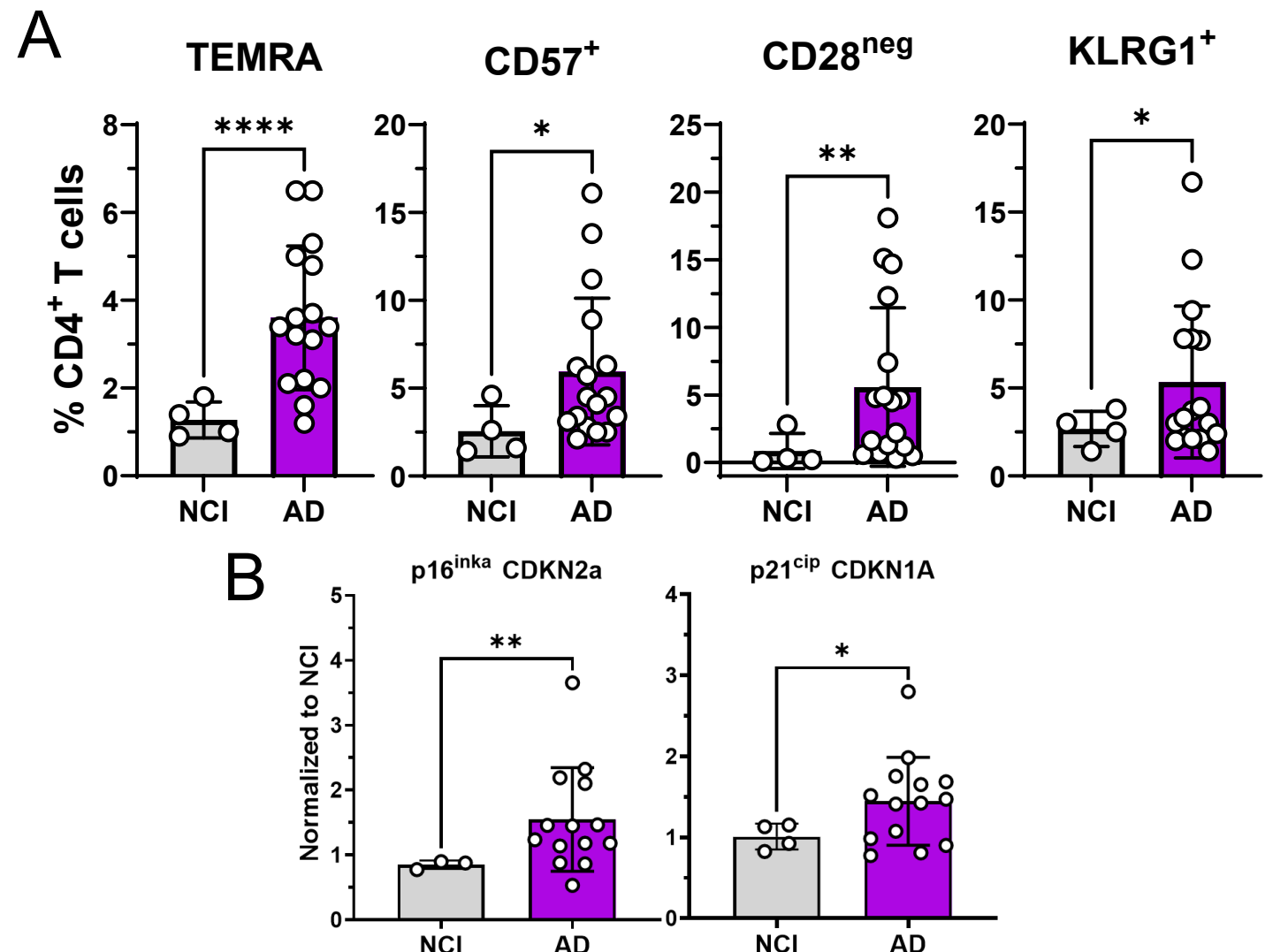
Fig 1. Gating strategy to assess circulating senescent and exhausted T cell phenotypes in AD and NCI



PBMC were stained with panel of fluorescent mAb to assess CD4 and CD8 T cells and their senescent (CD57⁺ or CD28^{neg}) or exhausted (KLRG1⁺ or PD1⁺) phenotypes. (A) CD3⁺ T cells were gated for CD4⁺ or CD8⁺ subsets. (B) CD4⁺ T cells stained for naïve/memory phenotypes. (C) Scheme defining where naïve, central memory (Tcm), effector memory (Tem) and effector T cells (Teff), and TEMRA fall in the analysis. (D) Gated total CD4⁺ T cells expressing senescent (CD57⁺, CD28^{neg}) and exhaustion (KLRG1⁺, PD1⁺) phenotypes.

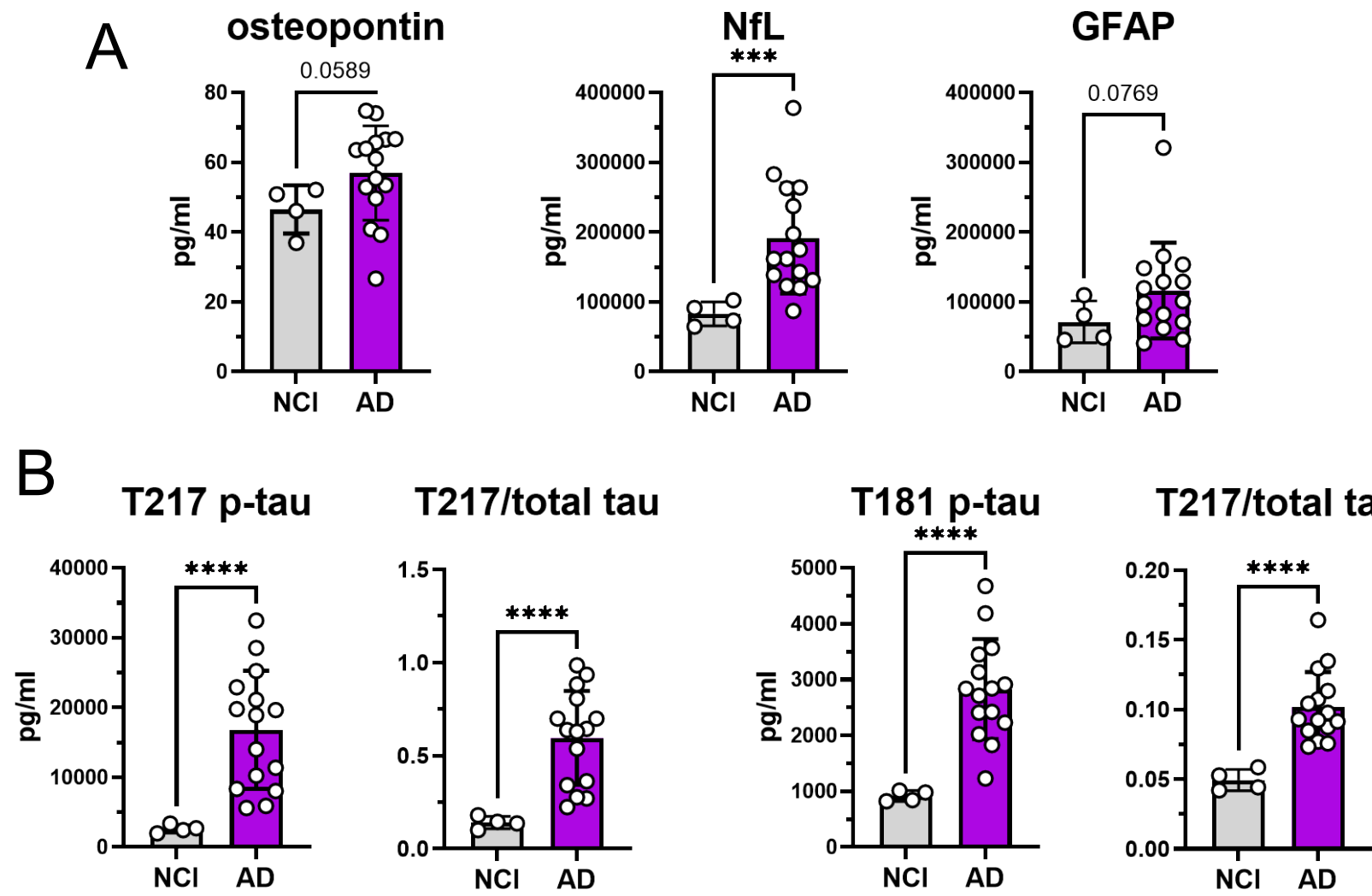
RESULTS

Fig 2. Senescent and exhausted CD4⁺ T cells are increased in AD PBMC



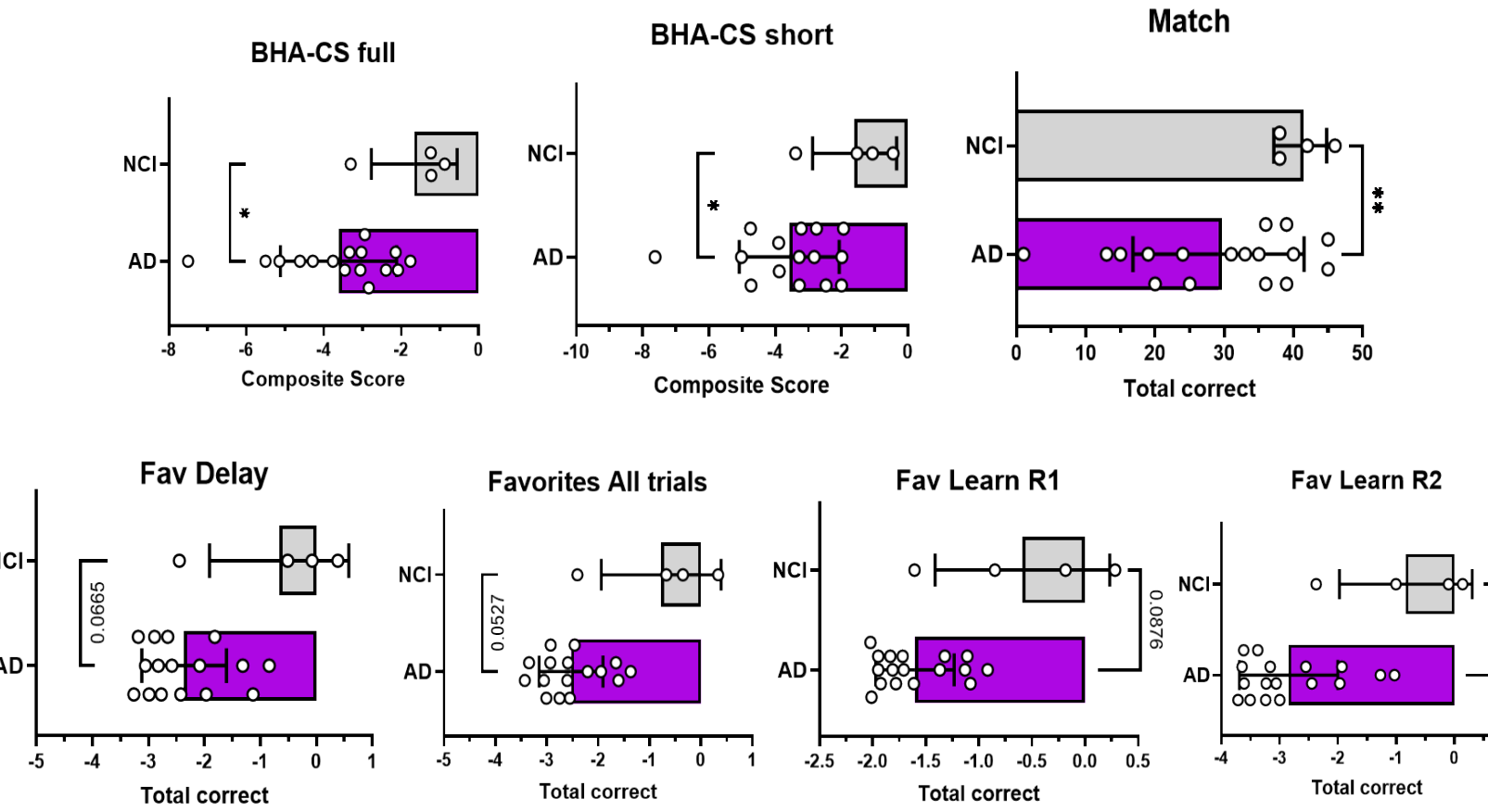
(A) Summary of flow cytometric analysis of CD4⁺ T cell phenotypes associated with senescence (TEMRA, CD57⁺ CD28^{neg}) or exhaustion (KLRG1⁺) that are increased in the PBMC of AD cohort compared to age-matched NCI. (B) qPCR analysis shows that the senescence-associated cyclin-dependent kinase inhibitors, p16^{INKa} and p21^{CIP} are increased in PBMC of AD cohort. Unpaired Welch’s t test analyses indicate significance <0.05 (*), <0.01 (**), <0.0001 (****) as indicated.

Fig 3. Plasma markers of neuroinflammation and phospho-tau species are increased in AD cohort



Using MesoScale Discovery assays for (A) neuroinflammation or (B) total tau and phospho-tau species, plasma levels of neurofilament light chain (NfL) were shown to be significantly increased and osteopontin and GFAP trended higher. Phospho-tau at T217 and T181 were also significantly increased. Unpaired Welch’s t test analyses indicate significance <0.001 (***) or <0.0001 (****) as indicated.

Fig 4. Cognitive and memory differences in AD cohort over NCI individuals as measured by TabCAT



TabCAT scoring was significantly lower in AD versus NCI individuals. Unpaired Welch’s t test analyses indicate significance <0.05 (*) or <0.01 (**) as indicated. Trending relationships are indicated with p < 0.1 > 0.05.

Table 2. Correlation analysis between frequency of senescent or exhausted CD4⁺ T cells in PBMC and AD disease measures

	Correlations with CD4 ⁺ T cell phenotypes							
	TEMRA		CD28neg		CD57+		KLRG1+	
	Spearman r	effect	Spearman r	effect	Spearman r	effect	Spearman r	effect
	p value	direction	p value	direction	p value	direction	p value	direction
Osteopontin	0.458	trend↑	0.386	ns	0.427	trend↑	0.000	ns
	0.056		0.114		0.077		>0.999	
GFAP	0.108	ns	-0.056	ns	0.509	↑	0.238	ns
	0.669		0.826		0.031		0.341	
NfL	0.671	↑	0.388	ns	0.405	trend↑	-0.026	ns
	0.002		0.111		0.095		0.919	
ptau 217/total tau	0.613	↑	0.316	ns	0.456	trend↑	-0.020	ns
	0.007		0.202		0.057		0.938	
ptau181/total tau	0.611	↑	0.451	trend↑	0.382	ns	-0.063	ns
	0.007		0.060		0.117		0.803	
IFABP	0.006	ns	0.111	ns	0.266	ns	-0.375	ns
	0.981		0.660		0.286		0.125	
BHA-CS full	-0.395	trend↓	-0.429	trend↓	-0.103	ns	-0.258	ns
TabCAT	0.094		0.076		0.683		0.302	
FavDelay	-0.654	↓	-0.589	↓	-0.298	ns	-0.057	ns
TabCAT	0.002		0.008		0.215		0.817	
Fav_all trials	-0.503	↓	-0.619	↓	-0.168	ns	-0.023	ns
TabCAT	0.028		0.005		0.492		0.926	

Two-tailed, nonparametric Spearman correlation analyses were performed between the indicated peripheral CD4⁺ T cell populations and AD-associated disease measures. TEMRA and senescent CD4⁺ T cells directly correlated with neuroinflammatory and AD markers and indirectly correlated with memory scores as measured by TabCAT. Relationships were considered trending when p < 0.1 > 0.05, and significant when p < 0.05.

Conclusions

- Senescent CD4⁺ T cells are increased in the periphery of this AD cohort.
- Increased senescent CD4⁺ T cell frequency directly correlates with neuroinflammation and poorer performance on cognitive and memory tests.
- Assessment of naïve and memory CD4⁺ T cell phenotypes in the blood may be a useful tool for monitoring disease progression and severity.

Future Directions

- Single cell RNA sequencing analyses of PBMC will focus on CD4⁺ T cell phenotypes to elucidate helper cell differentiation, senescent phenotypes, and effector functions, prevalent in the AD cohort compared to age-matched NCI controls.

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