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Original Article

Phenotypic alterations in peripheral blood B Lymphocytes of patients with Alzheimer's Disease

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ABSTRACT

Introduction: Dysfunction of humoral immunity has been implicated in the pathogenesis of Alzheimer's disease (AD). The distribution of B lymphocyte subsets and their clinical relevance in AD remain unclear.
Objective: In this study, we aimed to investigate the distribution of peripheral blood B lymphocyte subsets and their relevance with cognition and biomarkers in AD.
Design, Setting, and Participants: We evaluated the immunophenotype of peripheral B lymphocytes in 27 AD patients confirmed by PET-Amyloid scan and 32 cognitively normal controls.
Results: The phenotype of B lymphocytes is altered in AD patients. AD patients exhibit a decrease in both the numbers and proportions of switched memory (SwM) B cells and double-negative (DN) B cells. The proportion of unswitched memory (USwM) B cells was increased after *in vitro* stimulation. Additionally, B cells that produce proinflammatory cytokines including GM-CSF, IFN-γ, and TNF-α are increased, while those that produce the anti-inflammatory cytokine IL-10 are decreased in AD patients after *in vitro* stimulation. These alterations in B cell populations were linked to cognitive functions and biomarkers, including Aβ42/40 and pTau181, in AD patients.
Discussion: This study reveals an altered B-lymphocyte phenotype in AD patients, marked by functional and compositional dysregulation. Further research incorporating mechanistic, longitudinal, and functional studies is needed to determine whether these immune perturbations directly contribute to AD pathogenesis or arise as secondary effects of neurodegeneration.

1. Background

Alzheimer's disease (AD) is the most common form of dementia and is characterized by progressive memory loss and cognitive impairment [1,2]. The pathological hallmarks of AD include extracellular amyloid-beta (Aβ) deposition and intracellular neurofibrillary tangles formed by hyperphosphorylated tau protein [3]. The immune system plays a crucial role in the pathogenesis of AD [4]. Microglia, the primary phagocytic cells in the brain, are responsible for the clearance of the Aβ and tau proteins [5,6]. A significant number of AD-associated risk genes identified through GWAS are linked to the immune system [7]. However, the phenotype and functional aspects of humoral immunity in AD remain incompletely understood.

Our previous studies reported a panel of autoantibodies that are involved in the pathogenesis of AD [8–11]. Other studies have also identified various autoantibodies in the circulation and cerebrospinal fluid of AD patients [12]. In the AD brain, many brain-reactive autoantibodies are associated with Aβ deposition [11,13,14], supporting an autoimmune hypothesis in AD [4]. Nevertheless, the mechanisms underlying the dysregulated autoantibody profile in AD have yet to be fully addressed. B lymphocytes, a key component of the adaptive immune system, not only function as antigen-presenting cells to activate T cells and regulate inflammatory responses but also play a pivotal role in humoral immunity by secreting autoantibodies [15]. It is not yet clear whether the phenotype of B cells is altered during the development of AD. In this study, we aimed to investigate the distribution of peripheral blood B lymphocyte subsets and their clinical relevance in AD.

2. Methods

2.1. Participants and clinical assessment

A total of 27 AD patients and 32 cognitively normal (CN) subjects were consecutively recruited from the Department of Neurology, Dap-

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ing Hospital. Subjects were excluded if they fulfilled any of the following conditions: (1) cardiac, pulmonary, hepatic, or renal failure, or any type of tumor; (2) autoimmune diseases that could have dysregulated B lymphocyte profiles; or (3) refused to participate. Written informed consent for participation and blood sampling was obtained from all participants and their legal representatives.

General cognitive function was first evaluated via the Mini-Mental Status Examination (MMSE). Individuals with MMSE scores lower than 27 were further subjected to a battery of neuropsychological tests, including the Clinical Dementia Rating (CDR), Montreal Cognitive Assessment (MoCA), Activities of Daily Living (ADL), and Hachinski Ischaemic Scale (HIS). The CDR scale was used to determine whether the participants were cognitively normal (CDR=0), had mild cognitive impairment (MCI) (CDR=0.5), or had dementia (CDR≥1). Participants with a CDR of 0.5 or higher further received an MRI scan, an amyloid-PET scan using a 18F-Florbetapir tracer, and a fluorodeoxyglucose (FDG)-PET scan to confirm the etiology of cognitive impairment. All participants were subjected to ApoE genotyping and blood tests, including blood cell count, fasting glucose, cholesterol, and hepatic and renal functions, vitamine B12, folic acid, and thyroxin levels. The diagnosis of AD was made according to the criteria of the National Institute on Aging and Alzheimer's Association (NIA-AA) [16,17]. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Daping Hospital.

2.2. Blood sample processing

Approximately 10 mL of blood sample was collected from each participant into an EDTA tube (Thermo Fisher Scientific, USA) and allowed to rest at room temperature for 30 min. The samples were subsequently centrifuged at 2000 × g for 10 min to separate the plasma from the blood cells. The blood cells were then diluted with phosphate-buffered saline (PBS) (Thermo Fisher Scientific, USA) and subjected to density gradient centrifugation via Ficoll to isolate peripheral blood mononuclear cells (PBMCs). The isolated PBMCs were cryopreserved in a solution of heat-inactivated fetal bovine serum and dimethyl sulfoxide (DMSO) (Merck Sigma, Germany) at a 9:1 ratio for subsequent analysis.

2.3. B lymphocyte phenotyping

The procedures for B lymphocyte phenotyping were carried out in accordance with a previous study [18]. Specifically, cryopreserved PBMCs from the AD and CN subjects were thawed and resuspended in flow cytometry buffer (FACS) (Thermo Fisher Scientific, USA). Cell viability was assessed via trypan blue (Solarbio, UK) staining, and live cells were resuspended at a concentration of 1×10^6 cells/mL in a 200 μL volume. The samples were then incubated with Human TruStain FcX (BioLegend, USA) to block Fc receptors, followed by staining with CD19-FITC, CD24-PE, CD27-APC–Cy7, CD38-APC, and IgD PE-Cy7 antibodies (BioLegend, USA) on ice for 30 min. Antibody titration was performed to determine the optimal concentration for accurate detection of positive cell populations. Following incubation, the samples were centrifuged, the supernatant was discarded, and the cells were washed with PBS before resuspension. Flow cytometry was conducted via a Sony flow cytometer, with lymphocyte populations identified on the basis of cell size (FSC) and granularity (SSC). A minimum of 20,000 gated events per sample were collected for analysis. The data were analysed via FlowJo software version 10.8.1.

2.4. Definition of B lymphocyte subtypes

CD19-positive cells were identified as B lymphocytes, and their subpopulations were further classified. B lymphocyte subtypes were distinguished via the use of CD27 and IgD markers [19,20]. Naïve B cells were characterized as $CD19^+CD27^-IgD^+$ cells. Double-negative (DN) B cells were identified as $CD19^+CD27^-IgD^-$ cells. Unswitched memory (USwM) B cells were identified as $CD19^+CD27^+IgD^+$ cells, whereas switched memory (SwM) B cells were identified as $CD19^+CD27^+IgD^-$ cells. Further staining with CD24 and CD38 antibodies allowed the identification of regulatory B cells (Bregs) as $CD19^+CD24^{high}CD38^{high}$ and plasmablasts (PBCs) as $CD19^+CD24^-CD38^+$ cells. The analysis strategy, performed via FlowJo version 10.8.1 software, is depicted in Fig. 1A.

2.5. Identification of B lymphocyte subtypes after stimulation

PBMCs from 17 randomly selected AD patients and CN subjects were cultured to stimulate cytokine production. Isolated PBMCs cultured at a concentration of 1×10^6 cells/mL were stimulated with 10 μg/mL CpG ODN (InvivoGen, France) and 1 μg/mL CD40 L (BioLegend, USA) in 1 mL of Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum (Thermo Fisher Scientific, USA) and 100 IU/mL penicillin–streptomycin for 43 h. Next, the cells were cultured for an additional 5 h with 10 ng/mL PMA and 200 ng/mL ionomycin (BioLegend, USA). After collection, Human TruStain FcX (BioLegend, USA) was added to block Fc receptors.

The cells were then divided into two groups and incubated on ice for 30 min with different antibody combinations: one group was stained with CD19 FITC/IL-6 PE/TNF-α PE-Cy7/GM-CSF APC/IFN-γ APC–Cy7 (BioLegend, USA), while the other group was stained with CD19 FITC/CD24 PE/CD27 APC–Cy7/CD38 PerCP/IgD PE-Cy7/IL-10 APC antibodies (BioLegend, USA). Since IL-10, IL-6, TNF-α, GM-CSF, and IFN-γ are intracellular markers, after being stained with extracellular antibodies, the cells were fixed and permeabilized with cell-fast Fix/Perm Solution (BioLegend, USA) before being stained with intracellular antibodies. All the antibodies were titrated according to the recommended concentrations.

2.6. Detection of biomarkers of neurodegeneration

We used the Fujirebio Lumipulse instrument to detect plasma biomarkers, including pTau181, Aβ40, and Aβ42. The Lumipulse instrument was set up according to the manufacturer's guidelines, the parameters and conditions were adjusted, and quality control was performed to ensure the accuracy of the results. Specific antibodies against pTau181, Aβ40, and Aβ42 were prepared and incubated with the samples before quantification on the instrument. The data were analysed via Lumipulse software to calculate biomarker concentrations.

2.7. Statistical analysis

The demographic and clinical characteristics of the participants were summarized via descriptive statistics, with continuous variables described as means and standard deviations (SDs) and categorical data summarized as absolute frequencies and percentages. Comparative group p values were determined via independent samples t tests, Mann-Whitney U test, or $\chi 2$ test where appropriate. Total B lymphocyte counts were calculated by multiplying the proportion of B cells within the total lymphocyte population by the total lymphocyte count. Counts for distinct B cell subsets were derived by multiplying the proportion of each subset within the total B lymphocyte population by the total B lymphocyte count. Spearman correlation analyses were conducted to examine the correlation of different parameters with blood biomarkers and with cognitive functions, adjusting for age, sex, education, ApoE genotype, body mass index, hypertension, diabetes, and hyperlipidemia. All the statistical tests were two-tailed, and a P value < 0.05 was considered statistically significant.

3. Results

3.1. Participant characteristics

A total of 27 AD patients confirmed by amyloid-PET and 32 CN subjects were recruited in this study. Table 1 shows the sociocharacteristics

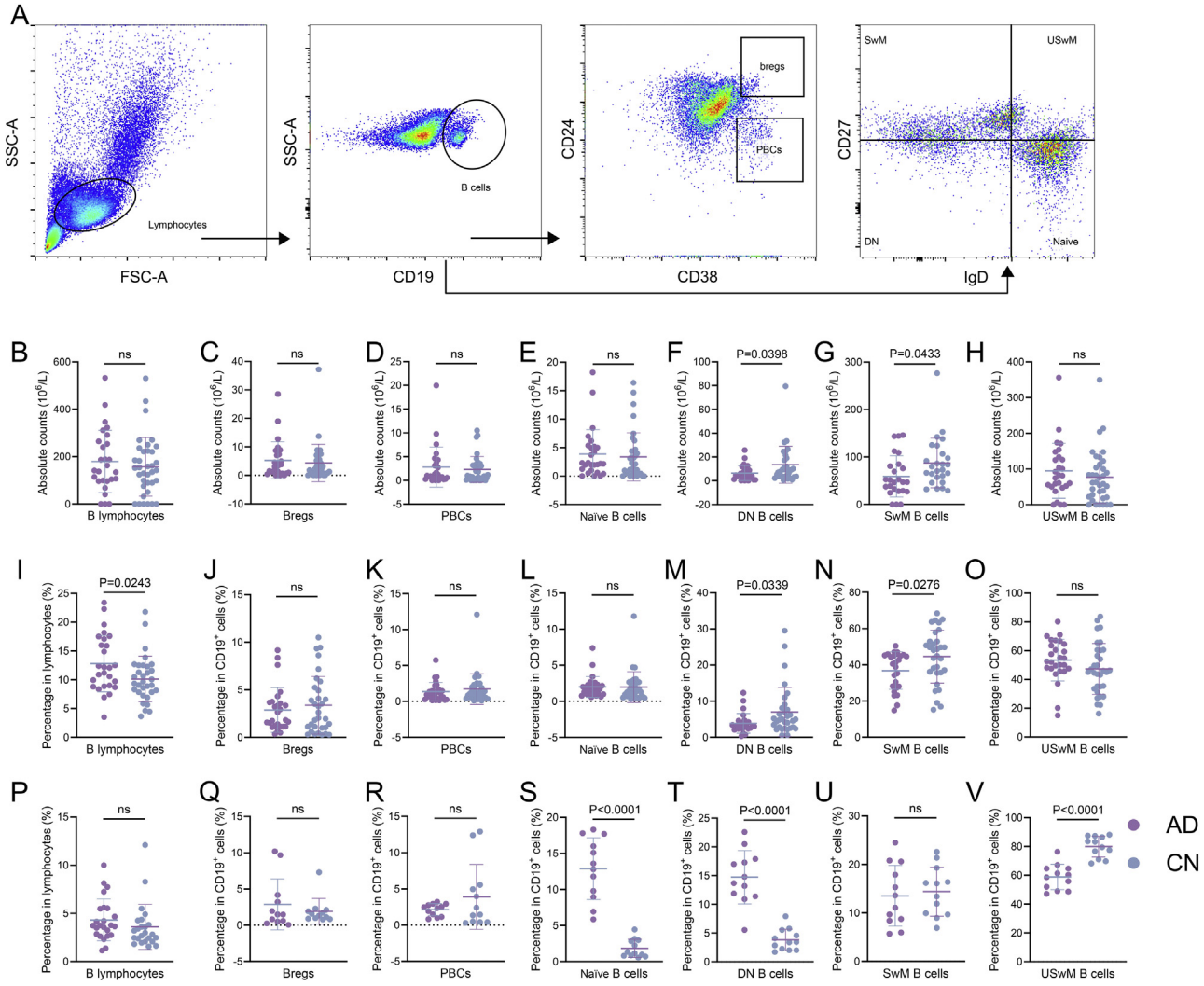

Fig. 1. B-lymphocyte phenotyping and distributions of different subsets.
(**A**) Identification process of B lymphocytes. Comparison of the absolute numbers of (**B**) total B lymphocytes, (**C**) Bregs, (**D**) PBCs, (**E**) naïve B cells, (**F**) DN B cells, (**G**) SwM B cells, and (**H**) USwM B cells between AD patients and CN subjects. Comparison of the percentages of (**I**) total B lymphocytes, (**J**) Bregs, (**K**) PBCs, (**L**) naïve B cells, (**M**) DN B cells, (**N**) SwM B cells, and (**O**) USwM B cells between AD patients and CN subjects. Comparison of the percentages of (**P**) total B lymphocytes, (**Q**) Bregs, (**R**) PBCs, (**S**) naïve B cells, (**T**) DN B cells, (**U**) SwM B cells, and (**V**) USwM B cells between AD patients and CN subjects after stimulation. Independent-sample t-test. The error bars represent standard deviation.

Table 1
Demographic characteristics of participants.

Variable	AD (n = 27)	CN (n = 32)	P value
Age, mean (SD), years	69.93 (9.68)	66.56 (6.20)	0.112
Sex			
Female, no. (%)	14 (51.85)	13 (40.62)	0.440
Male, no. (%)	13 (48.15)	19 (59.38)	0.440
Education, mean (SD), years	10.04 (3.07)	9.22 (3.11)	0.315
Body Mass Index, mean (SD)	22.88 (2.48)	24.26 (3.18)	0.07
ApoE ε4 carriers, no. (%)	6 (22.22)	4 (12.50)	0.488
Hypertension, no. (%)	7 (25.93)	9 (28.13)	>0.99
Diabetes Mellitus, no. (%)	7 (25.93)	7 (21.88)	0.766
Hyperlipidemia, no. (%)	3 (11.11)	4 (12.50)	>0.99
MMSE scores, mean (SD)	13.04 (8.08)	28.06 (2.12)	<0.001
CDR scores, median (IQR)	1 (0.5, 2)	0 (0, 0)	<0.001

The measurement data are represented by n (%); the numeration data are represented by the means ± SDs. ApoE ε4: apolipoprotein E ε4 allele, MMSE: Mini-Mental State Examination, CDR: Clinical Dementia Rating.

of the participants. There were no differences in mean age, sex distribution, mean education level, or body mass index between AD patients and CN subjects. Similarly, the proportion of ApoE ε4 carriers did not differ between the two groups. Additionally, the frequencies of hypertension, diabetes mellitus, and hyperlipidemia were comparable across the two groups. As expected, AD patients presented lower MMSE scores and higher CDR scores than did CN subjects (Table 1).

3.2. *Distribution of peripheral blood b lymphocytes and their subpopulations*

The absolute counts of total B lymphocytes were comparable between AD patients and CN subjects. AD patients presented lower absolute counts of DN B cells and SwM B cells than did CN subjects. The absolute counts of Bregs, PBCs, naïve B cells, and USwM B cells did not differ between the two groups (Fig. 1C, D, E, and H). The percentages of total B lymphocytes were greater in AD patients than in CN subjects. The percentages of SwM B cells and DN B cells were decreased, whereas those of USwM B cells were increased in AD patients (Fig. 1M–O). There

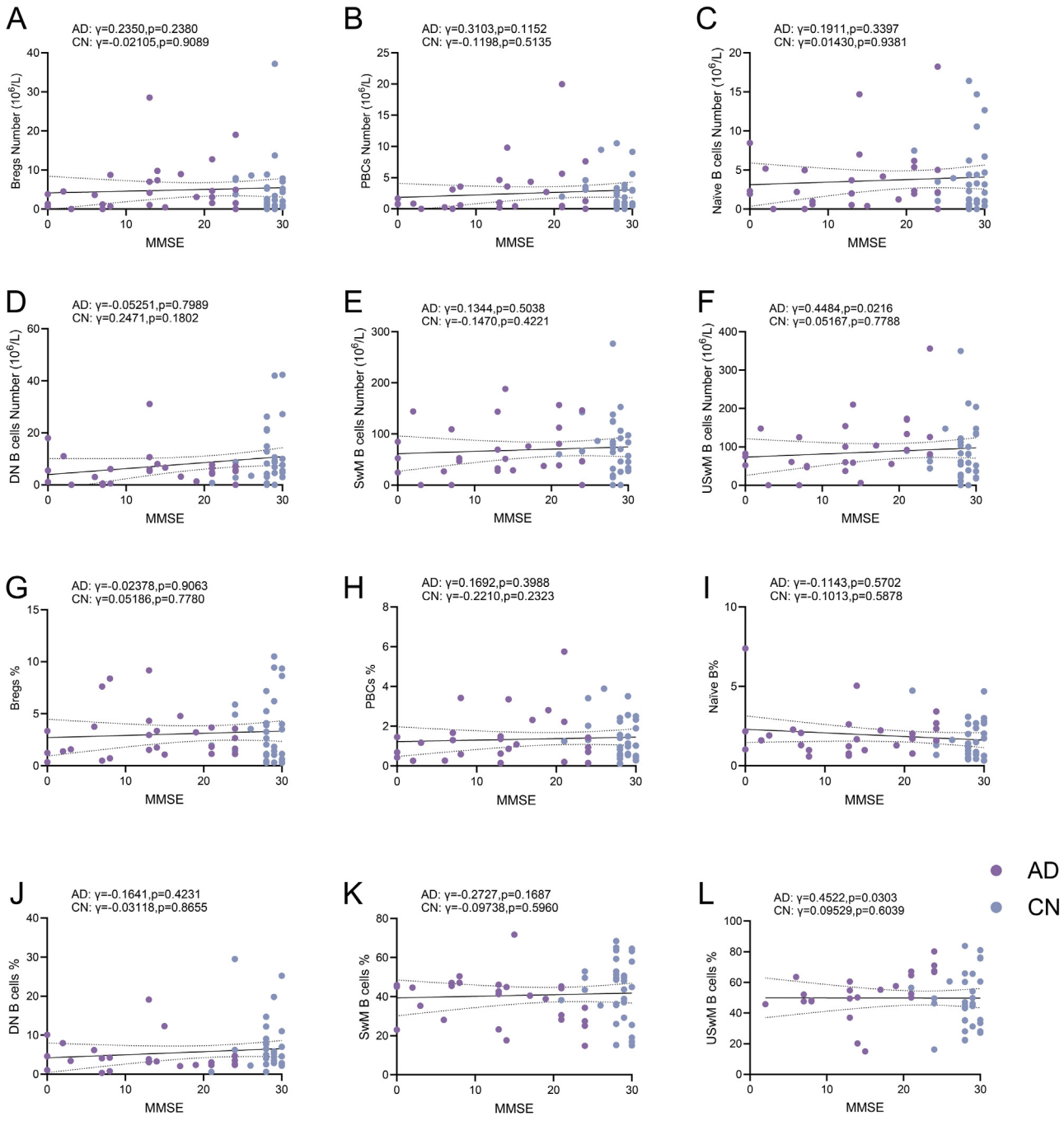

Fig. 2. Associations of peripheral blood B lymphocyte subpopulations with cognitive function.
Correlations of the absolute numbers of (**A**) Bregs, (**B**) PBCs, (**C**) **naïve** B cells, (**D**) DN B cells, (**E**) SwM B cells, and (**F**) USwM B cells with MMSE scores. Correlations of the percentages of (**G**) Bregs, (**H**) PBCs, (I) **naïve** B cells, (**J**) DN B cells, (K) SwM B cells, and (**L**) USwM B cells with MMSE scores. Spearman correlation analysis. The solid line reprents the regression line and dotted lines represent the 95 % confidence intervals, respectively.

were no significant differences in the percentages of Bregs, PBCs, or naïve B cells between AD patients and CN subjects (Fig. 1J-L).

3.3. Associations of peripheral blood B lymphocyte subpopulations with cognitive functions and biomarkers of AD

We explored the associations of different B lymphocyte subpopulations with cognitive functions and biomarkers of AD. The counts of Bregs, PBCs, naïve B cells, DN B cells, and SwM B cells showed no significant correlation with MMSE scores in either AD patients or CN subjects (Fig. 2A-E). However, the number of USwM B cells demonstrated a positive association with MMSE scores in AD patients, with no such relationship observed in CN individuals (Fig. 2F). Similarly, the percentages of Bregs, PBCs, naïve B cells, DN B cells, and SwM B cells also lacked correlation with MMSE scores across both groups (Fig. 2G-K). Notably, the percentage of USwM B cells exhibited a positive correlation with MMSE scores in AD patients, but not in CN subjects (Fig. 2L).

We next explored the associations between the percentages of B lymphocyte subpopulations and biomarkers of AD. The percentage of Bregs was positively associated with Aβ42/40 but negatively associated with pTau181 in the AD group (Fig. 3A and G). The percentage of PBCs was positively associated with Aβ42/40 in the AD group but not in CN subjects (Fig. 3B). However, the percentage of PBCs exhibited no significant correlation with pTau181 levels in either the AD or CN groups

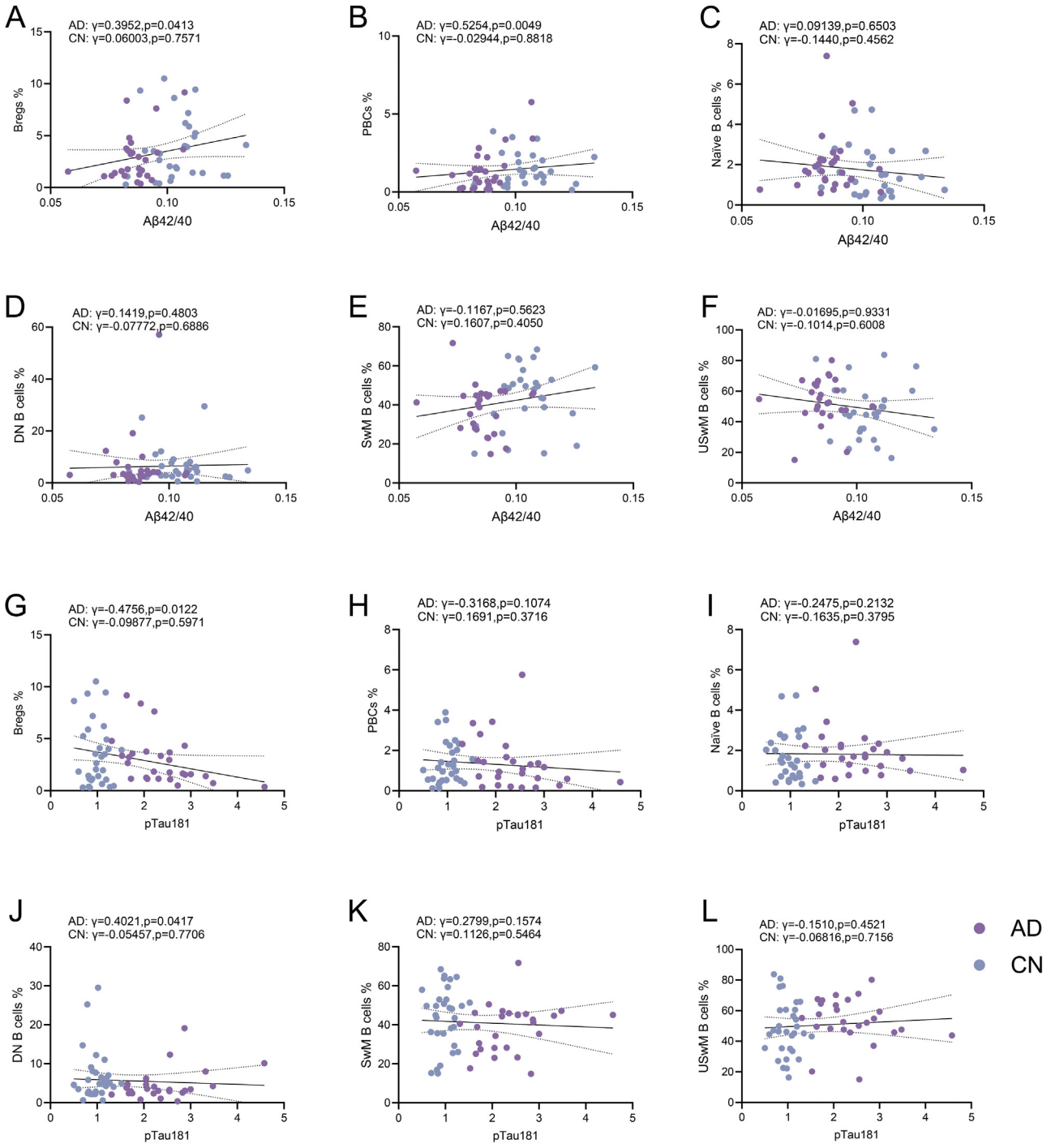

Fig. 3. Associations of peripheral blood B lymphocyte subpopulations with biomarkers of AD.
Correlations of the proportions of (**A**) Bregs, (**B**) PBCs, (**C**) **naïve** B cells, (**D**) DN B cells, (**E**) SwM B cells, and (**F**) USwM B cells with Aβ42/40. Correlations of the proportions of (**G**) Bregs, (**H**) PBCs, (**I**) **naïve** B cells, (**J**) DN B cells, (**K**) SwM B cells, and (**L**) USwM B cells with pTau181. Spearman correlation analysis. The solid line reprents the regression line and dotted lines represent the 95 % confidence intervals, respectively.

(Fig. 3H). Furthermore, the percentages of naïve B cells, DN B cells, SwM B cells, and USwM B cells showed no association with Aβ42/40 ratios or pTau181 levels (Fig. 3C-E and I-L).

3.4. Changes in B lymphocyte phenotypes after stimulation

To detect changes in B lymphocyte phenotypes after stimulation, PBMCs were cultured *in vitro* with CpG ODN, CD40L, PMA, and ionomycin and then analysed via flow cytometry after staining with the corresponding antibodies. We observed dramatic changes in the subpopulations of B lymphocytes after stimulation. The percentages of naïve B cells and DN B cells were significantly increased after stimulation in the AD group (Fig. 1S and T). The percentage of USwM B cells significantly decreased after stimulation. The percentages of total B lymphocytes, Bregs, PBCs, or SwM B cells did not differ between AD patients and CN subjects after stimulation (Fig. 1P, Q, R, and U).

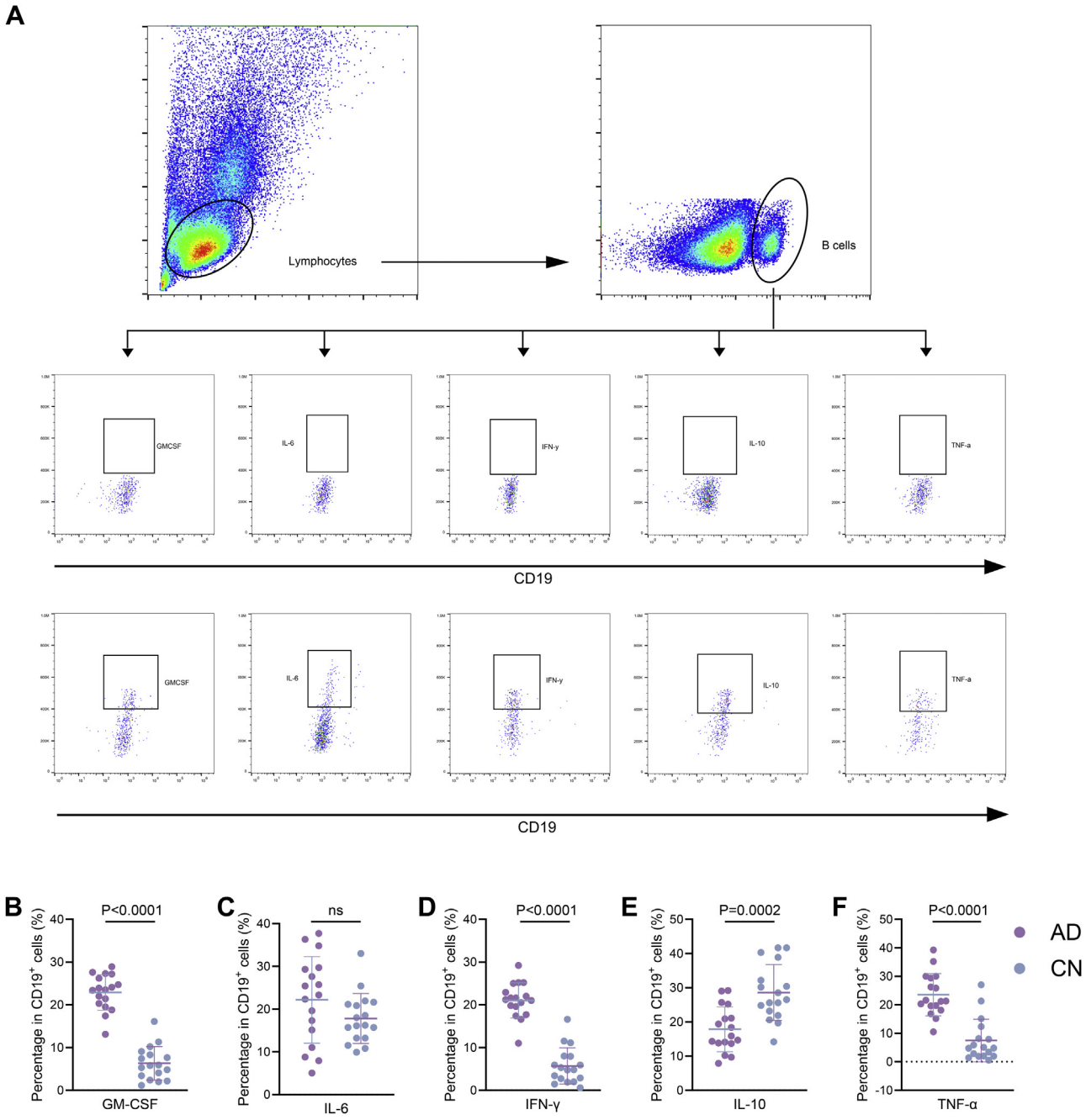

Fig. 4. Changes in B lymphocyte phenotypes after stimulation.
(**A**) Phenotyping and identification of cytokine-producing subsets of B lymphocytes after stimulation. (**B-F**) The percentages of cytokine-producing subsets of B lymphocytes after stimulation. Independent-sample t-test. The error bars represent standard deviation.

3.5. Changes in cytokine-producing subsets of B lymphocytes after stimulation

To investigate the cytokine secretion phenotype of B lymphocytes, we analysed the changes in cytokine-producing subsets of B lymphocytes after stimulation, including cells that produce GM-CSF, IL-6, IFN-γ, IL-10, and TNF-α. The proportions of B cells that produce GM-CSF, IFN-γ, and TNF-α were greater in AD patients than in CN subjects after stimulation (Fig. 4B, D and F), indicating a proinflammtory phenotype. However, the proportion of B cells that produce IL-10 was lower in AD patients than in CN subjects after stimulation (Fig. 4E).

3.6. Associations of cytokine-producing subsets of b lymphocytes with cognitive functions and biomarkers of AD after stimulation

After stimulation, the associations of B-cell-activating factors with cognitive functions and biomarkers of AD intensified. The proportions of B lymphocytes that secreted GM-CSF, IFN-γ, and TNF-α were negatively associated with cognitive functions in the AD group, but not in the CN group (Fig. 5A, C, and E). In contrast, the proportion of B lymphocytes that secret IL-10 was positively associated with the MMSE score in the AD group, but not in the CN group (Fig. 5D). After stimulation, the proportions of B lymphocytes that secreted GM-CSF, IFN-γ, and TNF-α were negatively correlated with Aβ42/40 levels but positively correlated

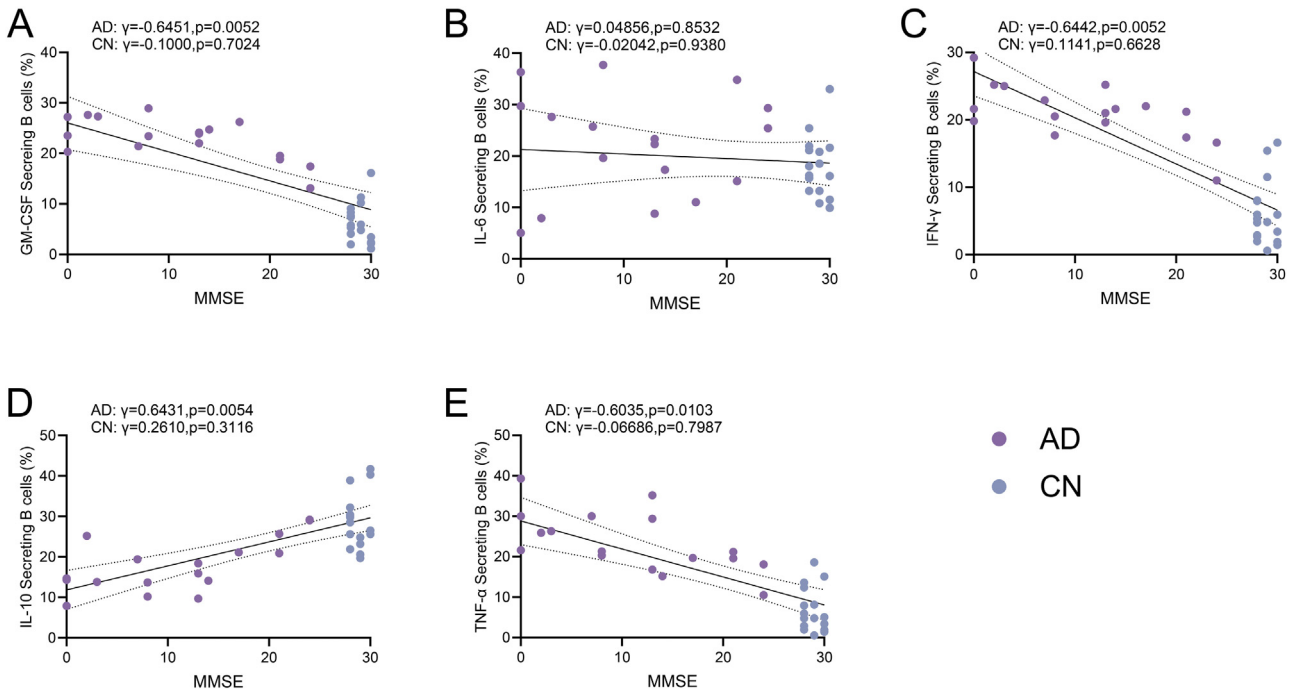

Fig. 5. Correlation of the percentage of cytokine-producing subsets of B lymphocytes with cognitive function after stimulation.
Correlations of the percentages of (**A**) GM-CSF-producing B cells, (**B**) IL-6-producing B cells, (**C**) IFN-γ-producing B cells, (**D**) IL-10-producing B cells, and (**E**) TNF-α-producing B cells with MMSE scores after stimulation. Spearman correlation analysis. The solid line reprents the regression line and dotted lines represent the 95 % confidence intervals, respectively.

with pTau-181 levels in the AD group, but not in the CN group (Fig. 6A, C, and E, and Fig. 6F, H, and J). Conversely, B lymphocytes secreting IL-10 were positively associated with Aβ42/40 levels but negatively associated with pTau-181 levels in the AD group, but not in the CN group (Fig. 6D and I).

4. Discussion

In this study, we identified distinct phenotypic alterations in B lymphocyte subpopulations among individuals diagnosed with AD. Overall, AD patients demonstrated a more activated state of B lymphocytes. Specifically, there was a decrease in numbers and proportions of SwM B cells and DN B cells, whereas the proportion of USwM B cells were unchanged in AD patients. Upon stimulation, B lymphocytes in AD patients transformed into a more proinflammatory state, represented by the increase in proportions of B cells that produce GM-CSF, IFN-γ, and TNF-α, and the decrease in the proportion of B cells that produce IL-10. These changes in B cell phenotype were clinically relevant to cognitive functions and biomarkers associated with AD.

Previous studies have shown conflicting results regarding B lymphocyte counts in AD patients, with some reporting an increase [21] and others reporting a decrease [22–25]. In this study, we found that there was an increase in the relative ratio of B lymphocytes but not in the number of B lymphocytes in AD patients. B-cell depletion has been shown to reduce Aβ plaques and reverse behavioral memory deficits [26]. However, these findings primarily reflect the systemic impact of total B cell depletion on AD pathogenesis; the phenotypic and functional contributions of individual B cell subtypes remain poorly characterized. We detected a decrease in DN B cells and SwM B cells in AD patients.

Bregs play an immunosuppressive role by secreting anti-inflammatory cytokines such as IL-10. Mice deficient in IL-10 exhibit accelerated experimental autoimmune encephalomyelitis [27–29]. Some studies have shown that Bregs can protect against AD by inhibiting inflammatory responses [30]. Although the number and proportions of Bregs did not change, The percentage of Bregs were positively associated with Aβ42/40 and negatively associated with pTau181 in the AD group, supporting a protective role of Bregs in AD from a clinical perspective. PBCs have been shown to be increased in autoimmune diseases such as primary Sjögren's syndrome [31] and systemic lupus erythematosus [32]. In this study, we found no difference in either the number or the proportion of PBCs between AD patients and CN subjects. Naïve B cells circulate between lymphoid organs, monitoring the presence of homologous antigens as the first step of adaptive immunity. After recognizing an antigen, naïve B cells can be activated and differentiate into plasma cells to produce antibodies or memory B cells [33]. In this study, we also found no difference in the number or proportions of naïve B cells under physical conditions between AD patients and CN subjects. However, the proportion of naïve B cells increased after *in vitro* stimulation, indicating that naïve B lymphocytes are autoreactive and may play a pathogenic role in AD patients. DN B cells exhibit characteristics of memory B cells, which can differentiate into plasmablasts to secrete antibodies and promote cellular senescence [34]. Previous studies have shown that the number of DN B cells increased with aging [35,36]. DN B cells may represent exhausted memory B cells, and their increase in aged individuals could be related to a decline in immune functions [37]. In this study, although DN B cells were found to be decreased, their proportion dramatically increased after *in vitro* stimulation in AD patients. Repeated or prolonged infections or stimulation cause memory B cells to undergo class-switch recombination in germinal centers, resulting in more effective antibody production. SwM B cells produce IgG, IgA, and IgE, whereas USwM B cells produce IgM and IgD [38]. The number and proportion of SwM decreased, but the those of USwM B cells were unchanged in AD patients. This aligns with prior research suggesting that SwM B cells are associated with a lower risk of AD [39]. However, following stimulation, the proportion of SwM B cells no longer differed between AD patients and CN subjects, but the proportion of USwM B cells decreased markedly in AD patients. These findings suggest an aberrant immune response in AD, potentially driven by dysregulated B cell activation, survival, or functional pathways under stimulated

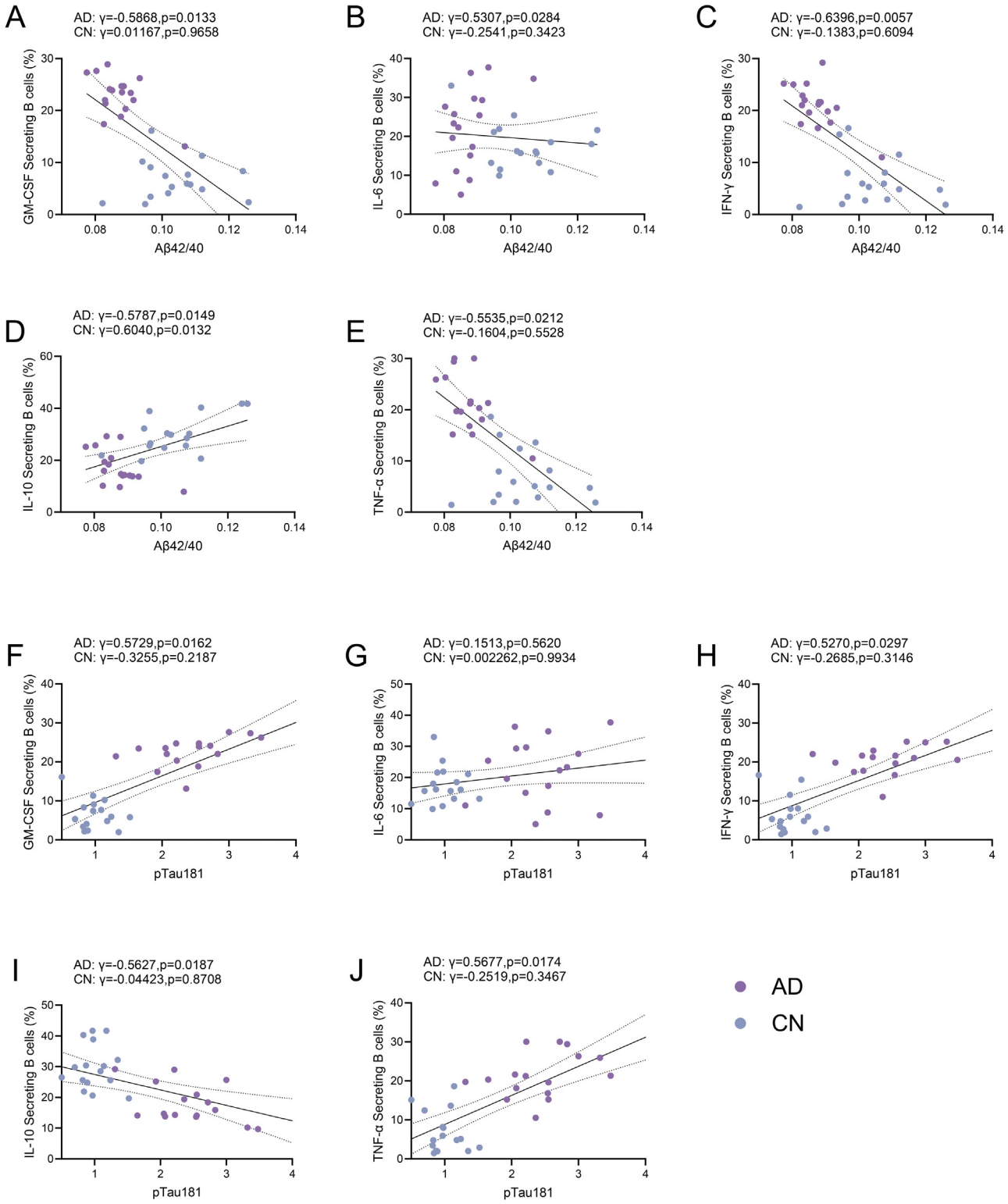

Fig. 6. Correlation of the percentage of cytokine-producing subsets of B lymphocytes with biomarkers of AD after stimulation.
Correlation of the percentages of (**A**) GM-CSF-producing B cells, (**B**) IL-6-producing B cells, (**C**) IFN-γ-producing B cells, (**D**) IL-10-producing B cells, and (**E**) TNF-α-producing B cells with Aβ42/40 after stimulation. Correlation of the percentages of (**F**) GM-CSF-producing B cells, (**G**) IL-6-producing B cells, (**H**) IFN-γ-producing B cells, (**I**) IL-10-producing B cells, and (**J**) TNF-α-producing B cells with Aβ42/40 after stimulation. Spearman correlation analysis. The solid line reprents the regression line and dotted lines represent the 95 % confidence intervals, respectively.

conditions. In support of these findings, we observed an increase in the proportions of B lymphocytes that secret proinflammatory cytokines such as GM-CSF, IFN-γ, and TNF-α, along with a reduction in the level of the anti-inflammatory cytokine IL-10 after stimulation. Moreover, stimulation-induced alterations in B-cell subpopulations and cytokine profiles were correlated with poorer cognitive performance and abnormal AD biomarker levels. This implies that dysregulated B-cell immune responses may contribute to AD pathogenesis by exacerbating neuroinflammation or disrupting immune homeostasis.

Overall, this study revealed an aberrant phenotype of peripheral B lymphocytes, characterized by heightened autoreactive potential and altered functional responses. However, as a descriptive clinical observational study, our findings highlight associations rather than causal mechanisms. Further investigations, including functional assays and mechanistic studies, are required to confirm the specific roles of distinct B-cell subpopulations in AD pathophysiology. Importantly, our work focused solely on B lymphocytes; changes in other immune populations, such as T cells and natural killer (NK) cells, remain unexplored. Future studies should explore the roles of different lymphocyte populations, including T cells and NK cells, in AD pathogenesis. Such efforts could uncover novel therapeutic targets to restore immune homeostasis and mitigate disease progression.

Consent statement

Written informed consent for participation and blood sampling was obtained from all participants and their legal representatives.

Ethical approval

This work is approved by the Institutional Review Board of Daping Hospital, Third Military Medical University.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Yan-Jiang Wang reports financial support was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Meng-Ting Wang: Methodology, Investigation. **Ye-Ran Wang:** Writing – original draft, Formal analysis. **Gui-Hua Zeng:** Resources. **Xiao-Qin Zeng:** Resources. **Zhang-Cheng Fei:** Resources. **Jia Chen:** Resources. **Jin Zhou:** Resources. **Xin-Peng Li:** Resources. **Zhi-Qiang Xu:** Writing – review & editing. **Yan-Jiang Wang:** Writing – original draft, Conceptualization. **Yu-Hui Liu:** Writing – original draft, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tjpad.2025.100135.

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