Yu *et al. Immunity & Ageing* (2024) 21:81
https://doi.org/10.1186/s12979-024-00486-5

Immunity & Ageing

RESEARCH Open Access

Peripheral CD4$^+$ T cell phenotype and brain microglial activation associated with cognitive heterogeneity in aged rats

Lian Yu[1†], Miao-Miao Liu[1†], Mei-Qi Guan[2], Rui Wang[1], Xiao-Rong Yang[3], Xiu-Min Zhang[1], Jing-Jing Wei[2], Shu-Fen Wu[2], Hong Gu[1], Qiang Fu[1], Jun-Hong Guo[1*] and Yan-Li Li[1*]

Abstract

Cognitive decline is a critical hallmark of brain aging. Although aging is a natural process, there is significant heterogeneity in cognition levels among individuals; however, the underlying mechanisms remain uncertain. In our study, we classified aged male Sprague–Dawley rats into aged cognition-unimpaired (AU) group and aged cognition-impaired (AI) group by using an attentional set-shifting task. The transcriptome sequencing results of medial prefrontal cortex (mPFC) demonstrated significant differences in microglial activation and inflammatory response pathways between the two groups. Specifically, compared to AU rats, AI rats exhibited a greater presence of CD86-positive microglia and major histocompatibility complex class II (MHC-II)-positive microglia, along with elevated inflammatory molecules, in mPFC. Conversely, AI rats exhibited a reduction in the percentage of microglia expressing CD200R and the anti-inflammatory molecules Arg-1 and TGF-β. Additionally, peripheral blood analysis of AI rats demonstrated elevated levels of Th17 and Th1 cells, along with proinflammatory molecules; however, decreased levels of Treg cells, along with anti-inflammatory molecules, were observed in AI rats. Our research suggested that peripheral Th17/Treg cells and central microglial activation were associated with cognitive heterogeneity in aged rats. These findings may provide a new target for healthy aging.

Keywords Aging-associated cognitive decline, Cognitive heterogeneity, Microglial activation, Th17/Treg cells, Inflammation

Introduction

With the aggravation of population aging, the problems associated with aging are becoming increasingly prominent. Cognitive decline is a significant characteristic of aging. It has been estimated that approximately 130 million elderly all over the world will experience aging-associated cognitive decline by 2050, which has become a serious public health problem [1].

Although aging is a universal phenomenon, there is significant heterogeneity in cognition levels among aged individuals [2]. Some individuals show aging-associated cognitive decline, whereas others are able to sustain optimal health and cognitive abilities as they age [3]. Several

†Lian Yu and Miao-Miao Liu contributed equally to this work.

*Correspondence:
Jun-Hong Guo
neuroguo@163.com
Yan-Li Li
liyanli_01@163.com
[1]Department of Neurology, Research Center for Neurological Diseases, First Hospital of Shanxi Medical University, Taiyuan 030001, China
[2]Department of Pediatrics, Shanxi Medical University, Taiyuan 030001, China
[3]The Department of Physiology, Shanxi Medical University, Taiyuan 030001, China

studies have reported the possible reason for such differences. For example, old participants with poor performance in memory measures only recruit the right prefrontal cortex (PFC), but old participants who perform well engage PFC regions bilaterally [4]. This general increase in prefrontal activation is an adaptive and compensatory alteration to counteract aging-associated neural decline [5]. Animal studies have also shown that cortical functional connectivity (FC) reduced significantly in aged cognition-impaired (AI) rats, while cortical FC is relatively preserved in aged cognition-unimpaired (AU) rats [6]. However, these imaging data still explain little about the mechanisms of cognitive differences in the elderly individuals.

Immunity and inflammation play important roles in aging and cognitive decline. $CD4^+$T cells mediate immunity in the periphery, including different types of cells like Th17, Th1, and Treg cells, and are considered key factors in aging [7]. Th17 cells have been demonstrated to exacerbate inflammation in different neurological disorders. In contrast, Tregs have anti-inflammatory effects and can inhibit Th17 cells, although they exhibit shared transcriptomic characteristics [8, 9]. Maintaining the equilibrium between Th17 and Treg cells, along with their corresponding transcription factors and cytokines, is essential. When Th17/Treg cells are imbalanced, circulating inflammatory cytokine levels can increase, thus leading to an inflammatory response. Upregulation of inflammatory cytokine levels in circulation enhances blood–brain barrier permeability and enables inflammatory cells and factors to enter brain tissue [10]. In brain, microglia are the main immune cells and can be activated by peripheral infiltrating T cells or inflammatory factors, thus releasing inflammatory factors and leading to neuroinflammation. Neuroinflammation can further damage neuronal and synaptic structures, thus affecting cognitive function [11]. Therefore, we speculate that microglial activation mediated by peripheral $CD4^+$T cells may be an important reason for aging-associated cognitive heterogeneity.

Research has demonstrated that aged rats display individual cognitive differences in attentional set-shifting tasks, thus providing a good tool for studying aging-associated cognitive decline [12]. Thus, this study mainly utilizes a behavioral attentional set-shifting task and analyzes the impact of $CD4^+$T cells in peripheral blood and microglial activation in brain on cognitive heterogeneity in aged rats, thereby providing new interventional targets for achieving healthy aging.

Materials and methods

Animals

Three-month-old young ($n=8$) and 22-month-old aged male Sprague–Dawley rats ($n=20$) were used in our study. They were purchased from Chengdu Dashuo Laboratory Animal Company in China. The rats were raised in Experimental Animal Center of Shanxi Medical University. The room temperature was set at approximately 22 °C ~ 25 °C. The relative humidity was set at 45% ~ 55%. The day–night cycle was maintained at 12/12 hours. All treatments given to the animals were approved by the Ethics Committee of the First Hospital of Shanxi Medical University.

Attentional set-shifting task based on operant conditioning

The attentional set-shifting task was performed as previously described [12]. In operant conditioning chambers (Med Associates, USA) as shown in Fig. 1A, rats learned to press the levers first, and then each rat's side bias was determined (more preferring the left lever or right lever). The task is formed in two stages (Fig. 1B). The first stage is visual cue discrimination. In this stage, the rats were

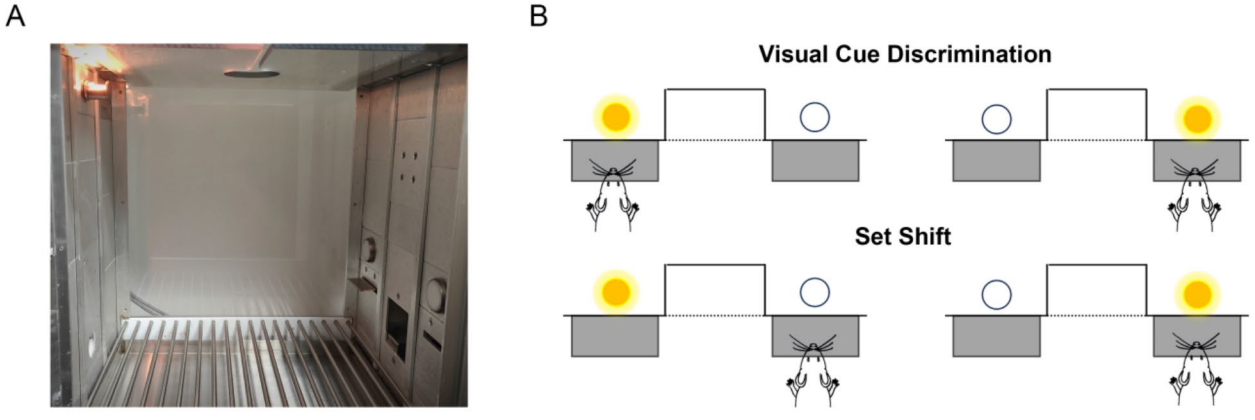

Fig. 1 Attentional set-shifting task based on operant conditioning. (**A**) Operant conditioning chambers. (**B**) Attentional set-shifting task program. It consists of two stages: the first stage (visual cue discrimination) and the second stage (set-shift)

trained to press the specific lever with visual cue light. At the beginning of each trial, a cue light on one side is randomly activated. After three seconds, the room light turns on, and both levers extend simultaneously. The lever aligned with the cue light is designated the correct choice. If the rat successfully presses the correct lever, it receives a reward, the signal light is extinguished, and the system prepares for the next trial. If the rat fails to select the correct lever or does not respond within ten seconds, the lever retracts, and both the room and signal lights turn off. A rat must complete eight consecutive trials correctly (with at least thirty total trials, excluding any omissions) to meet the criteria. Each rat can undergo up to 120 trials per day. Once the rats achieved the required criteria, they were then tested the following day in the second stage. The second stage is set-shift (left/right discrimination). In this stage, rats had to disregard the visual signal and consistently select either the left or right lever (the correct lever being the unbiased side that was defined during training). The setup and conditions remain identical to those in the visual cue recognition phase. When eight consecutive correct trials were achieved, the rats were determined to have achieved the set-shift criterion. The number of required trials to achieve the criterion was analyzed.

Golgi staining

It was performed according to the guidelines from the manufacturer (Hito Golgi-Cox OptimStainTM PreKit). Brain tissues were processed according to the procedure and then were cut into 100 μm-thick slices. The slices were stained and photographed at 40×, 200× and 1000× original magnifications by using TissueFAXS Plus S (TissueGnostics GMBH, Austria). The images were analyzed by using Image J software. And the spine number per 10 μm dendrite was calculated, that is the dendritic spine density. For quantification, 3 rats were used in each group, and 6 complete and clearly visible neurons were selected per rat.

Transcriptome sequencing

After rats were euthanized, brain tissues were collected, and medial prefrontal cortex (mPFC) was isolated. Total RNA was isolated via the TRIzol Reagent (Invitrogen Life Technologies), and its quality, integrity and concentration were subsequently assessed via a NanoDrop spectrophotometer (Thermo Scientific). For RNA sample preparation, three micrograms of RNA were utilized as the starting material. The sequencing libraries were constructed, and the library fragments were purified via the AMPure XP system (Beckman Coulter, Beverly, CA, USA). DNA fragments with adaptors ligated to both ends were selectively enriched via PCR via the Illumina PCR Primer Cocktail. The products were subsequently purified and quantified via the Agilent High Sensitivity DNA Assay on a Bioanalyzer 2100 system (Agilent). Finally, the sequencing libraries were subjected to sequencing on the Illumina NovaSeq 6000 platform at Shanghai Personal Biotechnology Company. Principal component analysis (PCA) was carried out based on gene expression data. The R package limma was used to identify differentially expressed genes (DEGs) on the basis of a linear model and modified *t* test. *P* values were adjusted for multiple testing with Benjamini–Hochberg correction. Based on the criteria of |log2FoldChange| > 1 and adjusted *P*-value < 0.05, differentially expressed genes (DEGs) were visualized in a volcano plot. We conducted GO functional enrichment analysis on DEGs using via the Metascape website. Terms with an adjusted *P*-value < 0.05 were considered significant. Gene set enrichment analysis (GSEA) was conducted on all detected genes utilizing the GSEA software provided by the Broad Institute. Gene set information was sourced from the GO biological process (BP) and KEGG databases. Terms with a *P*-value < 0.05 were deemed statistically significant.

Flow cytometry

As previously described, flow cytometry was utilized to detect peripheral T immune cells [13, 14]. Following the behavioral experiments, rats were anesthetized and the peripheral blood was taken. Mononuclear cells were isolated with lymphocyte separation solution (P8630, Solarbio), some of which were used for Treg detection, and the others were treated with a cell stimulation cocktail (00-4975-93, eBioscience) for 6 h and then used for the detection of Th1 and Th17 cells. First, antibodies against cell surface molecules, including anti-CD3-PerCP-Cy5.5 (201418, Biolegend), anti-CD4-FITC (11-0040-85, eBioscience) and anti-CD25-APC (17-0390-82, eBioscience), were used. Following a 30-minute incubation in darkness, the cells were fixed, permeabilized and then stained with antibodies against intracellular or nuclear factors, including anti-Foxp3-PE (12-4774-42, eBioscience), anti-IFN-γ-APC (50-7310-80, eBioscience) and anti-IL-17-PE (12-7177-81, eBioscience).

As previously described, flow cytometry was utilized to detect microglial activation in brain [15]. The mPFC tissues were digested with collagenase IV to prepare a single-cell suspension. After myelin was removed via gradient density centrifugation with 30% and 70% Percoll separation solution, the microglial cell layer was isolated. Purified anti-rat CD32 (550271, BD Biosciences) was added to block the Fc receptor. Afterwards, surface staining was performed with the following reagents: Zombie NIR™ Fixable Viability Kit (423105, Biolegend), anti-CD11b-PerCP-Cy5.5 (201819, Biolegend), anti-CD45-Pacific Blue (202225, Biolegend), anti-CD86-PE

(12-0860-83, eBioscience), anti-MHC-II-APC (17-0920-82, eBioscience), and anti-CD200R-FITC (204905, Biolegend).

The data were acquired via BD FACSCanto II flow cytometer (BD, USA) and then processed with FlowJo 10.8.1 software.

Enzyme-linked immunosorbent assay

Blood was centrifuged for collecting serum. The concentrations of IL-10 (JL13427, Jianglai Bio), IL-17 (JL20879, Jianglai Bio), IFN-γ (JL13241, Jianglai Bio) and TGF-β (JL13643, Jianglai Bio) in serum were analyzed according to the step-by-step instructions.

Real-time PCR

RNA of mPFC was extracted (9108Q, TakaRa), and concentration as well as purity of RNA were detected. Subsequently, the RNA was reverse transcribed to cDNA (RR047A, TaKaRa). And cDNA was then amplified by using real-time PCR master mix (RR820A, TaKaRa) and gene-specific primers. The LightCycler 480 II instrument (Roche Diagnostics GmbH, Germany) was used to conduct real-time PCR. The relative expression of a gene was calculated by $2^{-\Delta\Delta Ct}$. Four rats from each group were tested. The utilized primers: CD86 F: 5'-AGACATGTGTAACCTGCACCAT-3', R: 5'-ACTTTTTCCGGTCCTGCCAA-3'; IL-1β F: 5'-CAGCTTTCGACAGTGAGGAGA-3', R: 5'-TGTCGAGATGCTGCTGTGAG-3'; TNF-α F: 5'-CTCAAGCCCTGGTATGAGCC-3', R: 5'-GGCTGGGTAGAGAACGGATG-3'; IL-6 F: 5'-TCCTACCCCAACTTCCAATGC-3', R: 5'- TAGCACACTAGGTTTGCCGAG-3'; Arg-1 F: 5'-CCAGTATTCACCCCGGCTAC-3', R: 5'-GTCCTGAAAGTAGCCCTGTCT-3'; TGF-β F: 5'-GACCGCAACAACGCAATCTA-3', R: 5'-CGTGTTGCTCCACAGTTGAC-3'; and GAPDH F: 5'-GGCACAGTCAAGGCTGAGAATG-3', R: 5'-ATGGTGGTGAAGACGCCAGTA-3'.

Statistical analysis

The data analysis was carried out by using GraphPad Prism 9. All the data were normally distributed and had homogeneous variance; thus, the data were presented as means ± standard deviations ($\bar{X} \pm S$). Independent two-sample *t* tests were used to compare the differences between two groups. One-way analysis of variance (ANOVA) was applied for comparisons among multiple groups. *P* values < 0.05 were considered significant.

Results

Individual differences in the cognitive function of aged rats

Executive function is a sophisticated cognitive ability that regulates and integrates various other cognitive functions and behaviors, including attention, memory, and language. Numerous studies have demonstrated that cognitive flexibility, assessed through an attentional set-shifting task, can effectively indicate an individual's executive function. In our study, we utilized the attentional set-shifting task to measure cognitive flexibility. Fewer trials required to meet the criteria for the set-shifting task indicate superior cognitive flexibility, which, in turn, suggests better cognitive function. Compared with that of young rats, cognitive performance of aged rats was impaired, thus they needed more trials to achieve the desired performance level. Furthermore, the set-shifting performance of aged rats was more heterogeneous than that of young rats (Fig. 2A). With a standard deviation (SD) from the average performance in young rats as a control, the aged rats were divided into AU group and AI group. When the trials to criterion exceeded a SD from the average number in young rats, they were considered an AI rat ($n=9$). All of the other aged rats were AU rats ($n=11$). The cognitive performance of AI rats was significantly worse than that of young rats and AU rats ($P<0.01$, Fig. 2B).

The mPFC is the main brain area performing attentional set-shifting tasks in rodents. Dendritic spine density in the mPFC was observed via Golgi staining (Fig. 2C-F). Compared to that in AU group, the dendritic spine density in AI group was dramatically lower ($P<0.01$, Fig. 2E-F).

Transcriptome sequencing of the mPFC in AU and AI rats

RNA was isolated from the mPFC of AU and AI rats for transcriptome sequencing. Principal component analysis (PCA) demonstrated an obvious difference in gene expression between AU and AI rats (Fig. 3A). Compared with those of AU rats, the transcriptome profiles of the mPFC of AI rats exhibited distinct differences. A total of 139 DEGs were identified within the thresholds of |log2FoldChange| > 1 and adjusted *P* value < 0.05. Among these genes, 128 were upregulated, including Lpal2, Fcrl2, Gp2, Agpat2, Veph1, Aqp1, Efna4, and others. Conversely, 11 genes, including Sytl2 and Mis18a, were downregulated (Fig. 3B, Supplementary Table 1). Further GO analysis demonstrated that the DEGs were closely enriched in innate immune response, positive regulation of cytokine production and inflammatory response (Fig. 3C). Gene set enrichment analysis (GSEA) demonstrated that glial activation, microglial activation, the neuroinflammatory response and the TNF-mediated signaling pathway were upregulated in AI rats compared with AU rats (Fig. 3D). The gene expression associated with GSEA terms in the two groups of rats was visualized using a heatmap (Supplementary Fig. 1, Fig. 2).

Microglial activation differed between AU and AI rats

To assess microglial activation more specifically, flow cytometry was used to detect the marker for activated

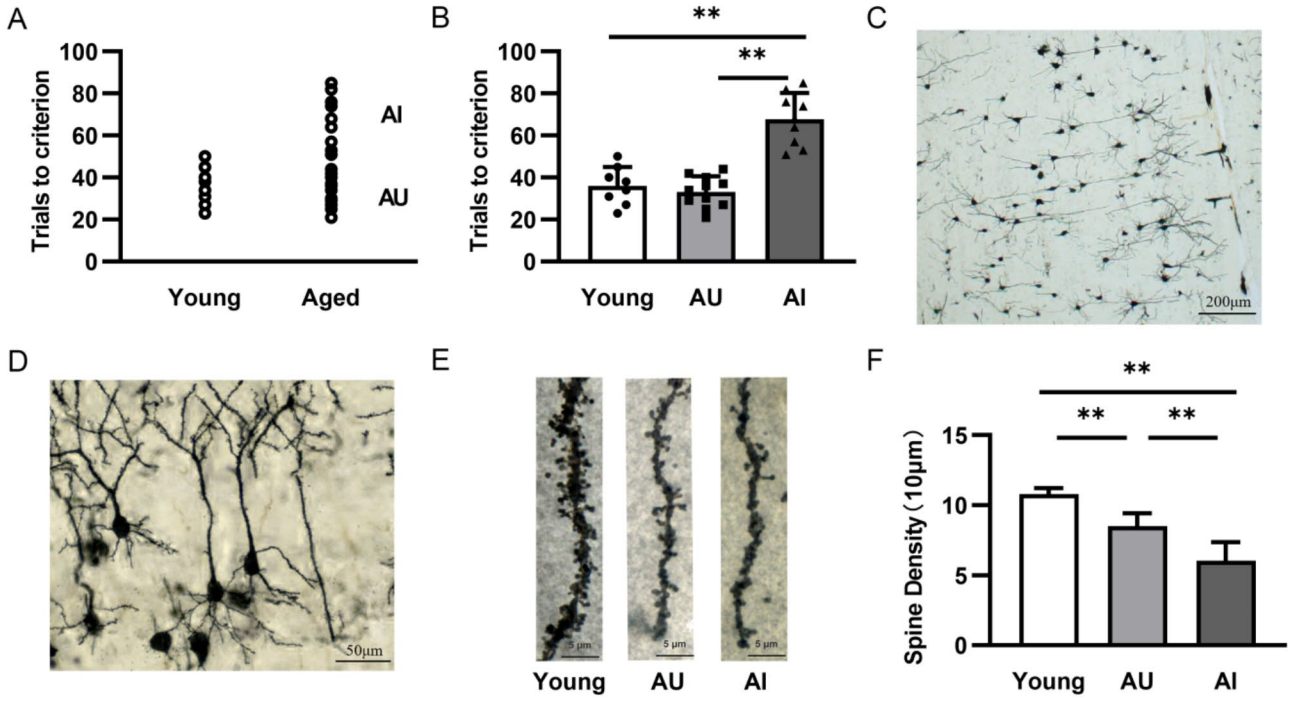

Fig. 2 Cognitive function in young and aged rats. (**A**) Cognitive performance in attentional set-shifting task. (**B**) Statistical analysis of the trials to criterion. Representative images of dendrites in the mPFC at 40× (**C**), 200× (**D**) and 1000× (**E**) magnification. (**F**) Statistical analysis of the dendritic spine density in each group. AU: aged cognition-unimpaired; AI: aged cognition-impaired. ** $P < 0.01$

microglia in the mPFC. CD86 and MHC-II have been reported as proinflammatory markers, while CD200R is recognized as an anti-inflammatory marker [16, 17]. Among microglia ($CD11b^{+}CD45^{int}$), there were more microglia expressing CD86, MHC-II and CD200R in both AI and AU rats than in young rats (Fig. 4). Compared with those in AU rats, the percentage of CD86-positive microglia was greater ($P<0.05$) and the percentage of MHC-II-positive microglia was greater ($P<0.01$), whereas the percentage of CD200R-positive microglia was lower in AI rats ($P<0.05$), suggesting an increase in inflammatory activity and a reduction in anti-inflammatory activity in AI rats.

Differential expression of central inflammatory factors in AU and AI rats

After microglia are activated, the expression of CD86 and MHC-II promotes neuroinflammation by increasing the secretion of inflammatory factors, thus resulting in aggravated neuroinflammation [18–20]. However, the expression of CD200R inhibits neuroinflammation and reduces the production of proinflammatory factors [21].

The expression of inflammation-related factors in mPFC was detected with RT-PCR (Fig. 5). Compared to those in AU rats, AI rats exhibited higher levels of proinflammatory markers CD86 ($P<0.01$), IL-1β, TNF-α and IL-6 ($P<0.05$), but lower levels of anti-inflammatory markers Arg-1 ($P<0.01$) and TGF-β ($P<0.05$).

The percentages of Treg, Th1 and Th17 cells in the peripheral blood of AU and AI rats were different

Among $CD4^{+}$ T cells ($CD3^{+}CD4^{+}$), $CD25^{+}Foxp3^{+}$ represents Treg cells, IFN-γ^{+} represents Th1 cells and IL-17^{+} represents Th17 cells. The percentages of Treg, Th1 and Th17 in $CD4^{+}$ T cells were greater in both AI and AU rats than in young rats. Compared to those in AU group, the percentage of Treg was lower ($P<0.01$), while the percentages of Th1 and Th17 were greater in AI group ($P<0.01$, Fig. 6).

Serum inflammatory factor levels differed between AU and AI rats

In the peripheral blood, Treg cells secrete anti-inflammatory IL-10 and TGF-β, and Th1 cells and Th17 cells secrete proinflammatory factors IFN-γ and IL-17 respectively. These factors in serum were detected by ELISA. In aged rats, IFN-γ and IL-17 increased (Fig. 7A, C, $P<0.01$), IL-10 and TGF-β also increased (Fig. 7E, G, $P<0.05$). Compared with those in AU rats, IFN-γ and IL-17 increased (Fig. 7B, D, $P<0.01$), whereas IL-10 decreased (Fig. 7F, $P<0.01$), and TGF-β decreased (Fig. 7H, $P<0.05$) in AI rats.

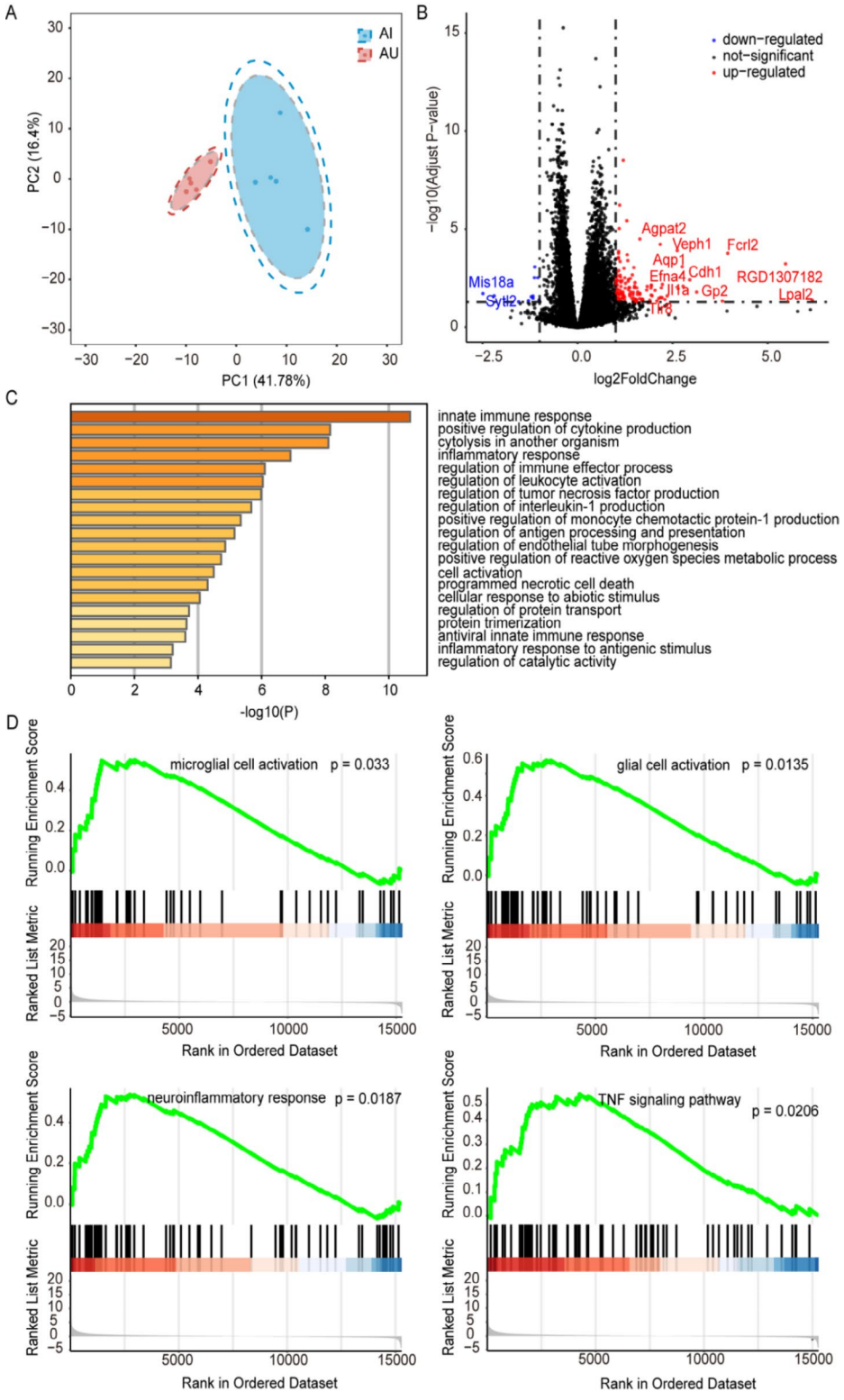

Fig. 3 Transcriptome difference analysis of the mPFC between AU and AI rats. (**A**) PCA plot. PC1, the first principal component; PC2, the second principal component. (**B**) Volcano plot of DEGs in each group. Y-axis: adjusted *P* value; x-axis: log2-fold change (log2FC). The red dots represent genes whose expression was upregulated more than log2(2), the blue dots represent genes whose expression was downregulated less than log2(0.5), and the black dots represent genes whose expression was not significantly different. (**C**) GO term enrichment analysis. x-axis: adjusted *P* value. (**D**) GSEA of all genes. *P* values < 0.05 were considered statistically significant

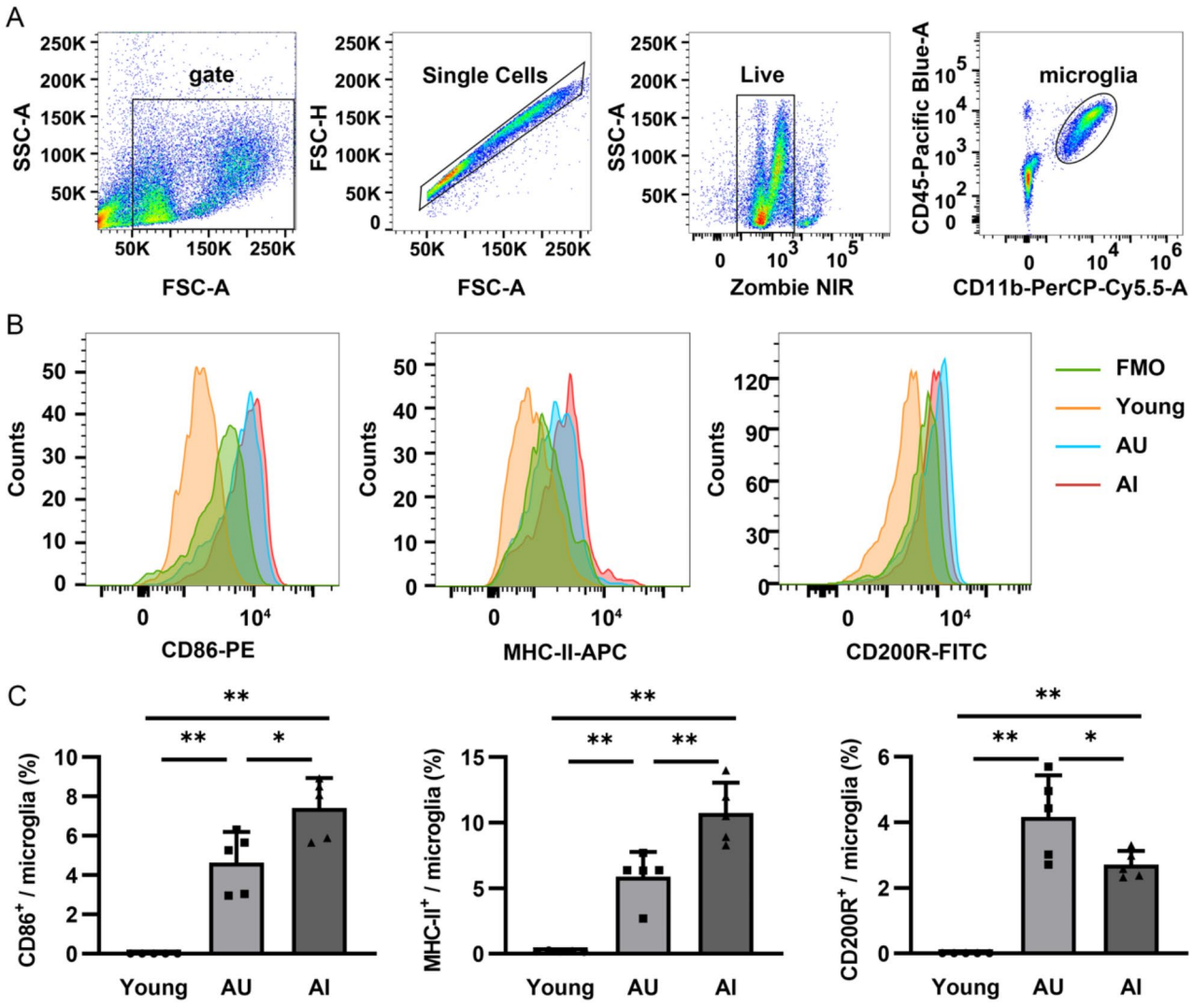

Fig. 4 Microglial activation was detected by using flow cytometry. (**A**) The gating strategy for microglia. The gate represents $CD11b^{+}CD45^{int}$-expressing microglia. (**B**) Flow cytometry plots showing CD86, MHC-II and CD200R expression on the surface of microglia. (**C**) The percentages of $CD86^{+}$, $MHC\text{-}II^{+}$ and $CD200R^{+}$ cells among the $CD11b^{+}CD45^{int}$ microglia. * $P < 0.05$, ** $P < 0.01$. FMO: fluorescence minus one control

Discussion

The mPFC is important for cognitive function in rodents [22]. Injury to the mPFC can affect cognitive flexibility. Cognitive flexibility refers to the ability to cope with sudden alterations in the environment by effectively updating internal representations and changing behavioral responses and is a part of executive function [23]. Previous studies on rats have shown that cognitive flexibility is highly susceptible to aging [24]. The attentional set-shifting task, which is an important indicator for evaluating cognitive flexibility, is sensitive to mPFC damage [25]. In this study, aged rats showed individual differences in attentional set-shifting task. Compared with performance of young rats, the AI and AU rats could be distinguished, which is consistent with the previous results [12]. The acquisition of initial rules remains unaffected, whereas the ability to adjust a shift is selectively impaired, thus reflecting the differences in mPFC functional activity between the two groups of rats [25]. To explore the mechanism of individual differences, transcriptome sequencing of the mPFC was performed. There were differences in the pathways associated with microglial activation and the inflammatory response between AU and AI rats.

Microglia, which are the main immune cells in brain, make up around 10% of brain cells [26]. Under physiological conditions, microglia have small cell bodies and maintain homeostasis of the nervous system by continuously monitoring the surrounding environment. When microglia are activated, their morphology rapidly

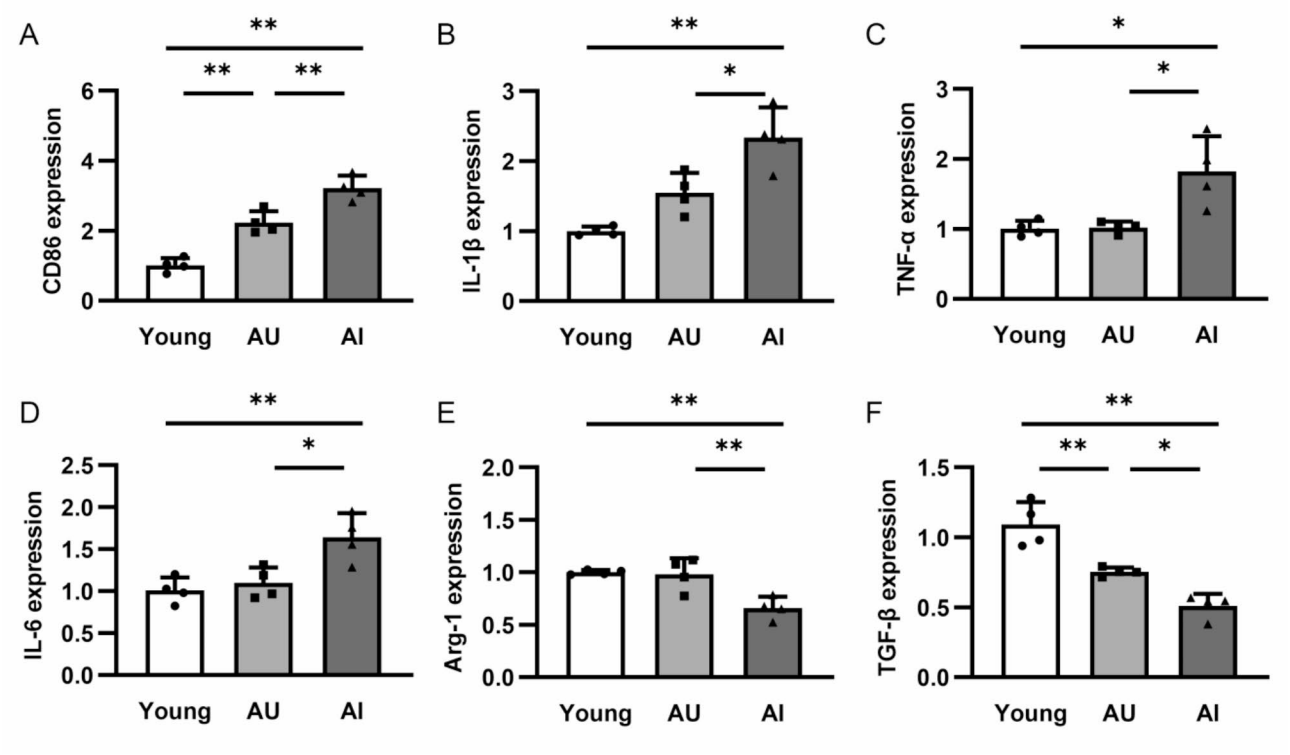

Fig. 5 The expression of central inflammatory factors in young, AU and AI rats. (**A**) CD86. (**B**) IL-1β. (**C**) TNF-α. (**D**) IL-6. (**E**) Arg-1. (**F**) TGF-β. * $P<0.05$, ** $P<0.01$

changes, which manifests as cell body enlargement; additionally, gene expression also changes [27]. During aging, microglia are activated, and express CD86 and MHC-II, which interact with T cells entering the brain to release inflammatory factors and exacerbate neuroinflammation [18–20]. CD200R on the surface of microglia is a receptor for CD200 [28]. Interaction with neuronal CD200 can not only inhibit microglial activation but can also suppress Ras-ERK and Ras-PI3K pathways to decrease inflammatory molecules [21]. In this study, compared with those in young rats, the number of microglia expressing CD86 or MHC-II increased in aged rats, which is consistent with previous research [29]. The expression of CD200R in the microglia with age also increased in an attempt to inhibit microglial activation and proinflammatory factor production. Interestingly, compared with those of AU rats, the mPFC of AI rats had more microglia expressing CD86 or MHC-II and fewer microglia expressing CD200R, which contributed to the increase in IL-1β, TNF-α and IL-6. The balance between different phenotypes of microglia is crucial in the aging process. During the early period of inflammation, the initial response of activated microglia is proinflammatory. Subsequently, anti-inflammatory microglia also increase to inhibit the inflammatory response, which is necessary for maintaining balance in the body [30]. However, during long-term chronic inflammation, microglia are continuously activated, and inflammatory factors continue to be produced, thus ultimately exacerbating neuroinflammation and cognitive impairment.

Immune dysregulation and inflammation in the circulation play important roles in aging. Compared with those in young individuals, $CD4^+$ T cells in elderly individuals produce more Th17 cell-related proinflammatory factors, including IL-6 and IL-17 A, which drive the body to exhibit an inflammatory state [31, 32]. However, inflammatory aging is not solely reflected in an increase in proinflammatory markers [33]. Treg cells are involved in the body's protection and can produce IL-10 to inhibit inflammation and prevent excessive immune responses [34]. Recently, single-cell RNA sequencing analysis from human peripheral blood showed the proportion of Tregs increased with age [35]. Similarly, in old mice, three $CD4^+$ T-cell subsets (exhausted, anti-inflammatory regulatory T cells, and proinflammatory cytotoxic T cells) gradually accumulate with age [36]. Consistent with these results, in our study, aged rats exhibited increased levels of proinflammatory Th17 and Th1 cells, as well as inflammatory molecules IL-17 and IFN-γ. Similarly, anti-inflammatory Treg cells, as well as anti-inflammatory factors IL-10 and TGF-β also increase with age, which reflects the body's adaptive response to proinflammatory stimuli that attempt to suppress inflammation [37]. More importantly, compared with AU rats, AI rats have more

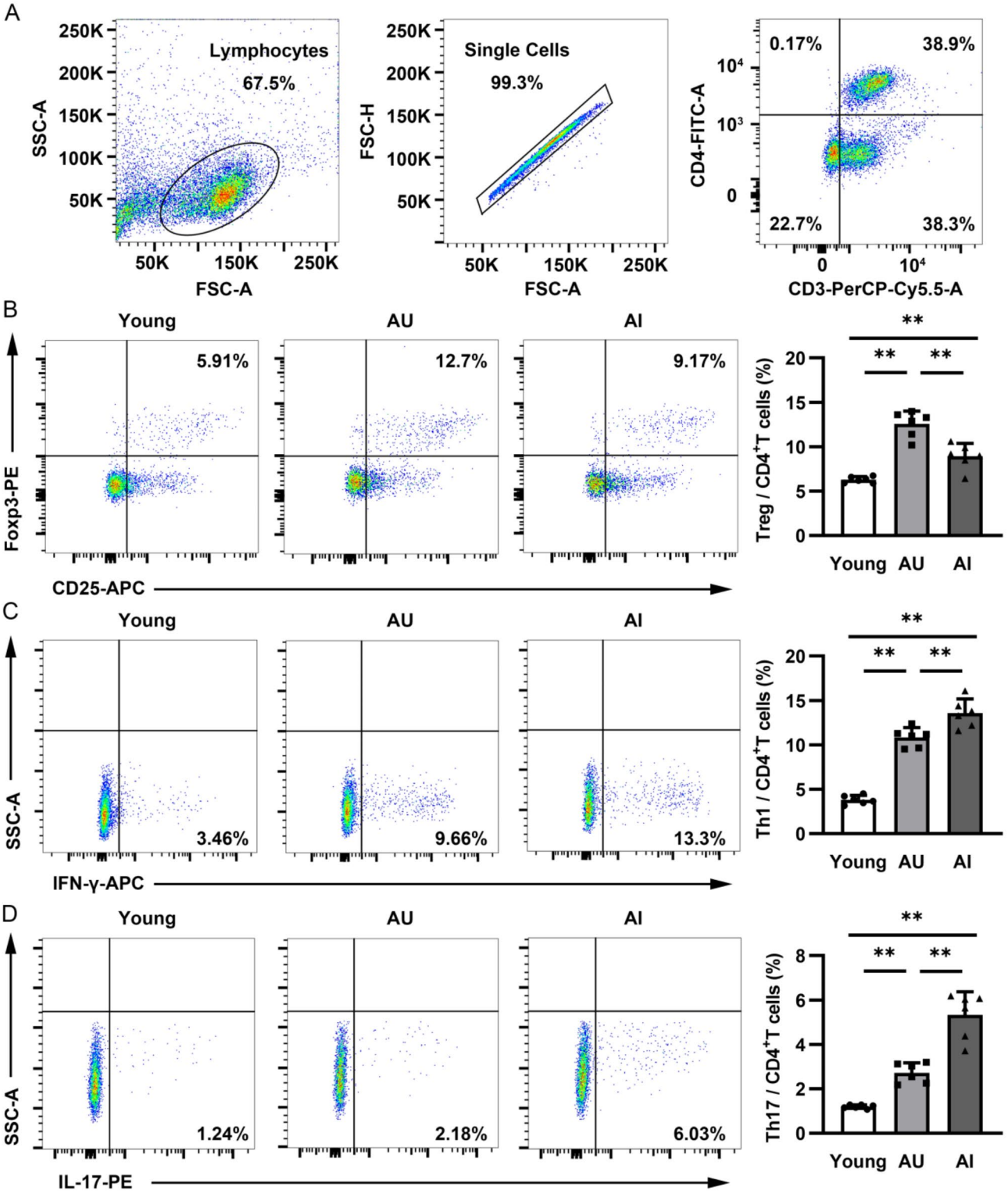

Fig. 6 The percentages of Treg, Th1 and Th17 in peripheral blood of young, AU, AI rats. (**A**) Gate strategy. The gate represents $CD4^+$ T cells. (**B**) Flow cytometry plots and the percentage of Treg. (**C**) Flow cytometry plots and the percentage of Th1. (**D**) Flow cytometry plots and the percentage of Th17. ** $P < 0.01$

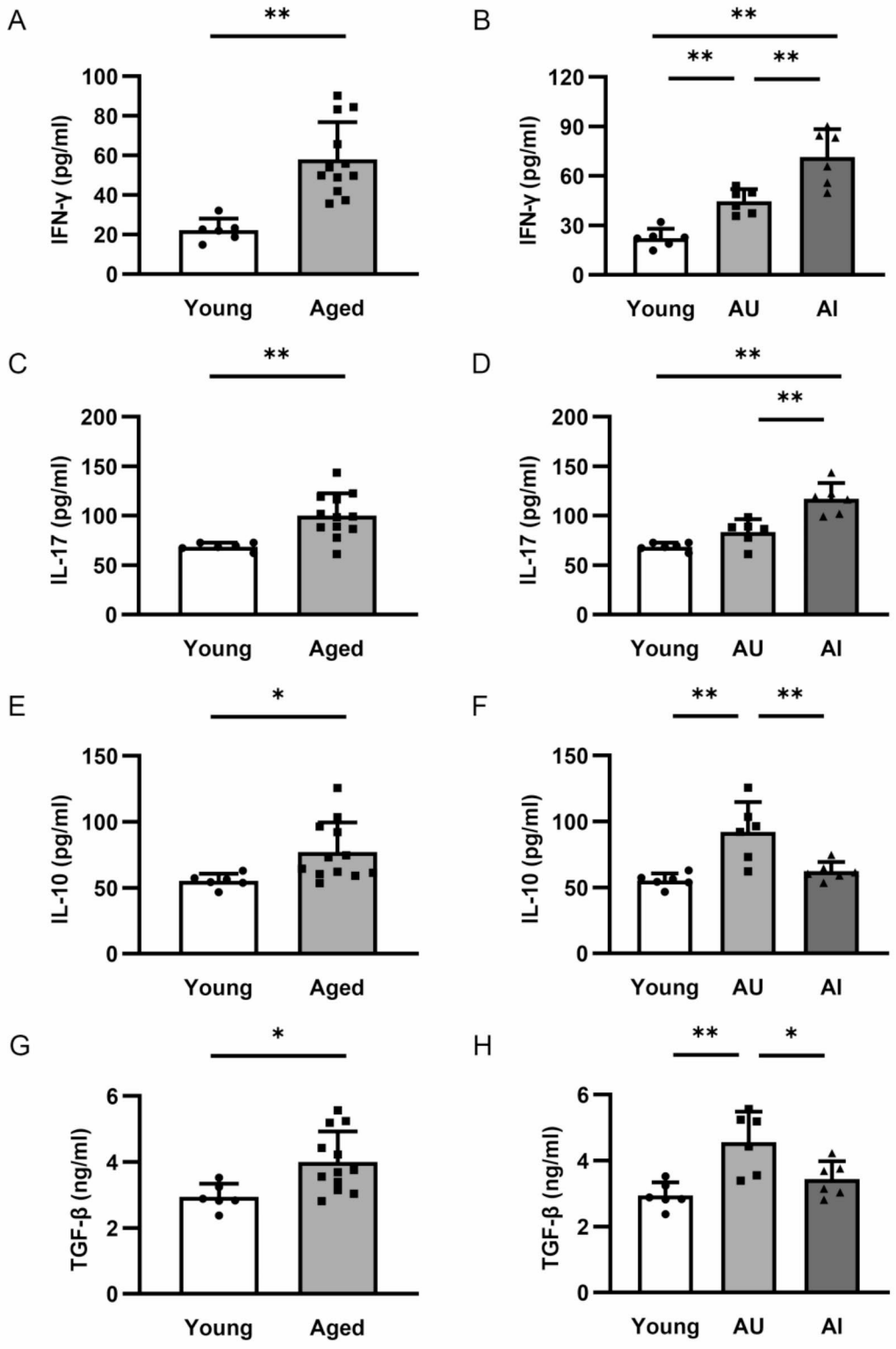

Fig. 7 The concentrations of inflammatory factors in serum. (**A**-**B**) IFN-γ. (**C**-**D**) IL-17. (**E**-**F**) IL-10. (**G**-**H**) TGF-β. * $P < 0.05$, ** $P < 0.01$

Th17 and Th1 cells, which produce more IL-17 and IFN-γ, whereas fewer Tregs as well as TGF-β and IL-10 are unable to suppress aging-related inflammation. This may be the reason for severe inflammation in AI rats.

Immune and inflammatory levels are key factors contributing to individual differences in the elderly individuals and can predict the development of aging-associated diseases. From an evolutionary perspective, inflammation has been regarded as an adaptation/remodel because it may activate anti-inflammatory responses to counteract aging-associated proinflammatory context. For example, centenarians have numerous anti-inflammatory factors in the circulation, such as IL-10, TGF-β1 and IL-1 receptor antagonists [38–40]. The anti-inflammatory state is activated to downregulate elevated inflammatory factors, such as C-reactive protein, IL-6 and IL-18 [41–43]. This implies that the immune system undergoes adaptive/maladaptive changes during the process of aging. The health of an individual depends on the adaptive/maladaptive consequences. It is determined by the ability to adapt to and reshape harmful stimuli [44].

In conclusion, chronic low-grade inflammation plays a significant role in aging-associated cognitive decline. Aging disrupts the equilibrium of peripheral Th17/Treg cells, thus resulting in elevated inflammation. Inflammation leads to heightened microglial activation in the mPFC, thus ultimately contributing to cognitive decline. Our study underscores the associations between peripheral and central immune states and aging-associated cognitive heterogeneity. Additionally, our research also indicates that healthy aging is not a sustained state of youthfulness but rather an adaptive reshaping of the body's aging state. The promotion of the Th17/Treg balance, rather than restoring it to a youthful state, may become a therapeutic target for aging-associated cognitive decline. Nevertheless, this study has several limitations. Currently, there is no detailed understanding of how $CD4^+$ T cells activate microglia, nor is it clear whether regulating the ratio of Th17/Treg can influence the activation of microglia. These questions urgently warrant further investigation and exploration. Moreover, to achieve a more precise analysis of the specific phenotypes and secreted factors associated with microglia, we recognize that the application of single-cell sequencing or spatial transcriptomics technologies would likely provide more accurate and detailed data. However, significant progress has yet to be made in this field.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12979-024-00486-5.

Supplementary Material 1

Acknowledgements
We would like to give thanks to all people participating in the study for their support and cooperation.

Author contributions
L.Y., M.L. and Y.L. conceived the study and designed the experiments. R.W., X.Y. and J.G. guided the experimental design. L.Y., M.L. and M.G. performed the experiments. R.W., X.Z., H.G. and Q.F. carried out data analysis. L.Y., M.L., X.Y. and S.W. carried out image processing. L.Y., M.L.and M.G. wrote the manuscript. J.W., J.G. and Y.L. reviewed and edited the manuscript.

Funding
This research was funded by National Natural Science Foundation of China, grant number 82101641; the Natural Science Foundation of Shanxi Province, China, grant number 20210302124173; Open Fund from Key Laboratory of Cellular Physiology (Shanxi Medical University), Ministry of Education, China, grant number KLMEC/SXMU-202010; National major R&D projects of China-Scientific technological innovation 2030, grant number 2021ZD0201801.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
The study was approved by Ethics Committee of First Hospital of Shanxi Medical University (DWLL-2023-006).

Competing interests
The authors declare no competing interests.

Received: 15 July 2024 / Accepted: 5 November 2024
Published online: 14 November 2024

References

1. Hay M, Barnes C, Huentelman M, Brinton R, Ryan L. Hypertension and age-related cognitive impairment: common risk factors and a role for Precision Aging. Curr Hypertens Rep. 2020;22:80.
2. Dekhtyar M, Papp KV, Buckley R, Jacobs HIL, Schultz AP, Johnson KA, et al. Neuroimaging markers associated with maintenance of optimal memory performance in late-life. Neuropsychologia. 2017;100:164–70.
3. Rowe JW, Kahn RL. Human aging: usual and successful. Science. 1987;237:143–9.
4. Cabeza R, Anderson ND, Locantore JK, McIntosh AR. Aging gracefully: compensatory brain activity in high-performing older adults. NeuroImage. 2002;17:1394–402.
5. Park DC, Reuter-Lorenz P. The adaptive brain: aging and neurocognitive scaffolding. Annu Rev Psychol. 2009;60:173–96.
6. Ash JA, Lu H, Taxier LR, Long JM, Yang Y, Stein EA, et al. Functional connectivity with the retrosplenial cortex predicts cognitive aging in rats. Proc Natl Acad Sci U S A. 2016;113:12286–91.
7. Le Page A, Dupuis G, Larbi A, W JM, T. F. Signal transduction changes in CD4(+) and CD8(+) T cell subpopulations with aging. Exp Gerontol. 2018;105:128–39.
8. Duffy SS, Keating BA, Perera CJ, Moalem-Taylor G. The role of regulatory T cells in nervous system pathologies. J Neurosci Res. 2018;96:951–68.
9. Lee GR. The balance of Th17 versus Treg cells in autoimmunity. Int J Mol Sci. 2018;19:730.
10. Wimmer I, Tietz S, Nishihara H, Deutsch U, Sallusto F, Gosselet F, et al. PECAM-1 stabilizes blood-brain Barrier Integrity and favors paracellular T-Cell diapedesis across the blood-brain barrier during Neuroinflammation. Front Immunol. 2019;10:711.
11. Zhao J, Bi W, Xiao S, Lan X, Cheng X, Zhang J, et al. Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. Sci Rep. 2019;9:5790.
12. Beas BS, Setlow B, Bizon JL. Distinct manifestations of executive dysfunction in aged rats. Neurobiol Aging. 2013;34:2164–74.

13. Sun X, He S, Lv C, Sun X, Wang J, Zheng W, et al. Analysis of murine and human Treg subsets in inflammatory bowel disease. Mol Med Rep. 2017;16:2893–8.
14. Loos J, Schmaul S, Noll TM, Paterka M, Schillner M, Löffel JT, et al. Functional characteristics of Th1, Th17, and ex-Th17 cells in EAE revealed by intravital two-photon microscopy. J Neuroinflammation. 2020;17:357.
15. Martin E, El-Behi M, Fontaine B, Delarasse C. (2017). Analysis of Microglia and Monocyte-derived macrophages from the Central Nervous System by Flow Cytometry. J Vis Exp:55781.
16. Walker DG, Lue LF. Immune phenotypes of microglia in human neurodegenerative disease: challenges to detecting microglial polarization in human brains. Alzheimers Res Ther. 2015;7:56.
17. Chávez-Galán L, Olleros ML, Vesin D, Garcia I. Much more than M1 and M2 macrophages, there are also CD169(+) and TCR(+) macrophages. Front Immunol. 2015;6:263.
18. Toledano Furman NE, Prabhakara KS, Bedi S, Cox CS Jr., Olson SD. OMIP-041: optimized multicolor immunofluorescence panel rat microglial staining protocol. Cytometry A. 2018;93:182–5.
19. Nguyen JN, Chauhan A. Bystanders or not? Microglia and lymphocytes in aging and stroke. Neural Regen Res. 2023;18:1397–403.
20. Poppell M, Hammel G, Ren Y. Immune Regulatory Functions of Macrophages and Microglia in Central Nervous System diseases. Int J Mol Sci. 2023;24:5925.
21. Mihrshahi R, Barclay AN, Brown MH. Essential roles for Dok2 and RasGAP in CD200 receptor-mediated regulation of human myeloid cells. J Immunol. 2009;183:4879–86.
22. Chong HR, Ranjbar-Slamloo Y, Ho MZH, Ouyang X, Kamigaki T. Functional alterations of the prefrontal circuit underlying cognitive aging in mice. Nat Commun. 2023;14:7254.
23. Dias R, Robbins TW, Roberts AC. Dissociation in prefrontal cortex of affective and attentional shifts. Nature. 1996;380:69–72.
24. Barense MD, Fox MT, Baxter MG. Aged rats are impaired on an attentional set-shifting task sensitive to medial frontal cortex damage in young rats. Learn Mem. 2002;9:191–201.
25. Floresco SB, Block AE, Tse MT. Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behav Brain Res. 2008;190:85–96.
26. Xu Y, Chen R, Zhi F, Sheng S, Khiati L, Yang Y, et al. δ-opioid receptor, Microglia and Neuroinflammation. Aging Dis. 2023;14:778–93.
27. Colonna M, Butovsky O. Microglia function in the Central Nervous System during Health and Neurodegeneration. Annu Rev Immunol. 2017;35:441–68.
28. Manich G, Recasens M, Valente T, Almolda B, González B, Castellano B. Role of the CD200-CD200R Axis during Homeostasis and Neuroinflammation. Neuroscience. 2019;405:118–36.
29. Frank MG, Barrientos RM, Biedenkapp JC, Rudy JW, Watkins LR, Maier SF. mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. Neurobiol Aging. 2006;27:717–22.
30. Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. Transl Neurodegener. 2020;9:42.
31. Bharath LP, Agrawal M, McCambridge G, Nicholas DA, Hasturk H, Liu J, et al. Metformin enhances autophagy and normalizes mitochondrial function to Alleviate Aging-Associated inflammation. Cell Metab. 2020;32:44–e5546.
32. Kroemer G, Zitvogel L. CD4(+) T cells at the Center of Inflammaging. Cell Metab. 2020;32:4–5.
33. Morrisette-Thomas V, Cohen AA, Fülöp T, Riesco É, Legault V, Li Q, et al. Inflamm-aging does not simply reflect increases in pro-inflammatory markers. Mech Ageing Dev. 2014;139:49–57.
34. Dikiy S, Rudensky AY. Principles of regulatory T cell function. Immunity. 2023;56:240–55.
35. Huang Z, Chen B, Liu X, Li H, Xie L, Gao Y, et al. Effects of sex and aging on the immune cell landscape as assessed by single-cell transcriptomic analysis. Proc Natl Acad Sci U S A. 2021;118:e2023216118.
36. Elyahu Y, Hekselman I, Eizenberg-Magar I, Berner O, Strominger I, Schiller M, et al. Aging promotes reorganization of the CD4 T cell landscape toward extreme regulatory and effector phenotypes. Sci Adv. 2019;5:eaaw8330.
37. Teissier T, Boulanger E, Cox LS. Interconnections between Inflammageing and Immunosenescence during Ageing. Cells. 2022;11:359.
38. Lio D, Scola L, Crivello A, Colonna-Romano G, Candore G, Bonafé M, et al. Inflammation, genetics, and longevity: further studies on the protective effects in men of IL-10 -1082 promoter SNP and its interaction with TNF-alpha – 308 promoter SNP. J Med Genet. 2003;40:296–9.
39. Carrieri G, Marzi E, Olivieri F, Marchegiani F, Cavallone L, Cardelli M, et al. The G/C915 polymorphism of transforming growth factor beta1 is associated with human longevity: a study in Italian centenarians. Aging Cell. 2004;3:443–8.
40. Cavallone L, Bonafè M, Olivieri F, Cardelli M, Marchegiani F, Giovagnetti S, et al. The role of IL-1 gene cluster in longevity: a study in Italian population. Mech Ageing Dev. 2003;124:533–8.
41. Gao D, Ni X, Fang S, Wang Z, Jiao J, Liu D, et al. Exploration for the reference interval of C-reactive protein in the Chinese longevity people over 90 years of age. Diabetes Metab Syndr. 2023;17:102817.
42. Bonafè M, Olivieri F, Cavallone L, Giovagnetti S, Mayegiani F, Cardelli M, et al. A gender–dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. Eur J Immunol. 2001;31:2357–61.
43. Gangemi S, Basile G, Merendino RA, Minciullo PL, Novick D, Rubinstein M, et al. Increased circulating Interleukin-18 levels in centenarians with no signs of vascular disease: another paradox of longevity? Exp Gerontol. 2003;38:669–72.
44. Fulop T, Larbi A, Hirokawa K, Cohen AA, Witkowski JM. Immunosenescence is both functional/adaptive and dysfunctional/maladaptive. Semin Immunopathol. 2020;42:521–36.

Publisher's note