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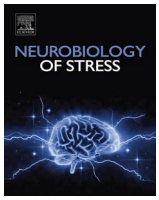

$CD4^+$ T-cell subsets are associated with chronic stress effects in newly diagnosed anxiety disorders

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ABSTRACT

Aim: Prior research has indicated a connection between $CD4^+$ T cells and the development of anxiety, but the specific $CD4^+$ T cell subsets linked to anxiety disorders remain uncertain. Our study seeks to investigate the relationship between distinct $CD4^+$ T cell subsets and anxiety, as well as to explore whether $CD4^+$ T cell subsets mediate the effect of chronic psychological stress on anxiety.

Methods: 56 eligible matched participants were recruited in Peking Union Medical College Hospital. The diagnosis was made based on DSM-5 diagnostic criteria. The severity of anxiety and depression symptoms was assessed using the Hamilton Anxiety Rating Scale and Hamilton Depression Rating Scale, respectively. The Life Events Scale (LES) evaluated the chronic stress level. $CD4^+$ T cell subsets were characterized using multiparametric flow cytometry. To assess the impact of $CD4^+$ T cells on the effect of chronic psychological stress on anxiety, Partial Least Squares Structural Equation Modeling (PLS-SEM) analysis was employed.

Results: We discovered fifteen notably distinct $CD4^+$ T-cell subsets in anxiety disorder patients compared to healthy controls. Multiple linear regression analysis unveiled an association between anxiety severity and $CD27^+CD45RA^-$ Th cells, $CD27^+CD28^+$ Tregs, and the total Life Events Scale (LES) score. The PLS-SEM analysis demonstrated that $CD4^+$ T cell subsets and LES could explain 80.2% of the variance in anxiety. Furthermore, it was observed that $CD27^+CD28^+$ Th/Treg cells acted as inverse mediators of the effects of LES on anxiety ($P = 0.031$).

Conclusions: Drug naïve anxiety disorder patients exhibited significant alterations in numerous $CD4^+$ T-cell subsets. Specifically, the memory subset of $CD27^+CD45RA^-$ Th cells and the naïve subset of $CD27^+CD28^+$ Treg cells were found to be independent factors associated with the severity of anxiety. Additionally, the $CD27^+CD28^+$ Th and Treg cell subsets played a significant mediating role in the influence of long-term psychological stress on anxiety.

1. Introduction

Anxiety disorders are common psychiatric disorders characterized by excessive and unnecessary worry and fear (Craske et al., 2017). In 2019, the estimated global prevalence of anxiety disorders was 301.4/million people, a disease burden second to that of depressive disorders("Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019," 2022). Panic disorder (PD) and generalized anxiety disorder (GAD) are two common types of anxiety disorders that significantly impair individuals' quality of life and functioning (Penninx et al., 2021). However, only 44.5% of anxiety disorders can be correctly detected depending on mental and somatic symptoms. Beyond that, the response rate to initial treatment in anxiety disorders is insufficient, with a rate of 45%–65% (Szuhany and Simon, 2022). Thus, it is essential to explore in-depth pathophysiological mechanisms of anxiety disorders to improve the efficacy in diagnosis and prognosis for anxiety disorders patients. Chronic and life event stress is considered a significant risk factor that leads to the occurrence of anxiety disorders (de Kloet et al., 2005). Moreover, numerous studies have revealed bidirectional interaction between chronic psychological stress and the immune system (Haroon et al., 2012; Leonard and Song, 1996; Nautiyal et al., 2008). Thus, detecting the immune mechanism of stress-linked psychiatric disorders, such as anxiety disorders, can provide a novel insight into

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comprehensively understanding the pathophysiology of anxiety disorders (Haykin and Rolls, 2021).

It has been established that chronic psychological stress can induce alterations in hematopoiesis and result in a pro-inflammatory process (Chan et al., 2023). Pro-inflammatory immune signaling is involved in regulating the hypothalamic-pituitary-adrenal (HPA) axis and other biological processes responsible for the modulation of affective behavior in response to stressor exposure (Haroon et al., 2012). Peripheral pro-inflammatory cytokines, such as IL-6 and IL-1β secreted by the intrinsic immune system, play critical physiological roles in the central nervous system, including the metabolism of neurotransmitters, neuroendocrine function, and effects on neuroplasticity, acting as neuroinflammatory factors in anxiety behaviors (Engler et al., 2017; McKim et al., 2018). Clinical studies revealed that serum levels of different cytokines, including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α, and INF-γ correlated with anxiety disorders (Michopoulos et al., 2017; Quagliato and Nardi, 2018; Tang et al., 2018). However, the changes in various cytokine levels in anxiety disorders are still inconclusive. A case-control study that included 20 patients with generalized anxiety disorder found that patients with generalized anxiety disorder have higher levels of TNF-α in the peripheral circulation and decreased levels of IL-2 and IL-4(Vieira et al., 2010). A more extensive cohort study showed that a down-regulated level of serum IL-1β, IL-2, and up-regulated IL-4 was observed in generalized anxiety disorders(Shen et al., 2022). The cohort study, including patients with generalized anxiety disorder, panic disorder, and phobias, found no significant difference in TNF-α levels in the patients compared to the healthy control (Vogelzangs et al., 2013). These results indicate that there must be heterogeneity of cytokine levels in anxiety disorders, which is partially due to variability in assay procedures and different study populations. Moreover, cytokines can also be produced by various immune cells and many other types of cells(Deets and Vance, 2021; Ortega et al., 2020). Therefore, it is necessary to explore another immune-related mechanism of anxiety disorder to develop robust biomarkers for diagnosis or prognosis prediction.

T cells have been demonstrated to be involved in modulating behavior, stress responsiveness, and memory (Serre-Miranda et al., 2015; Straub and Cutolo, 2018). $CD4^+$ T cells and $CD4^+CD25^+$ regulatory T cells have been investigated as subsets in neurogenesis and protection in the central nervous system (Evans, Dittmer, de la Fuente and Fitzgerald, 2019). Subsequently, more attention has been paid in recent years to the role of $CD4^+$ T cells in the onset of anxiety. Animal studies have shown that transferred $CD4^+$ T cells from stress-induced mouse to control can induce the generation of anxiety-like behavior (Rattazzi et al., 2013) by influencing the amygdala, which is part of the critical neural circuit for fear and anxiety-based disorders (Fan et al., 2019). Various immune dysfunction mouse models have also revealed that abnormalities in $CD4^+$ T cells but not $CD8^+$ T cells contribute to generating anxiety-like behavior through well-established brain networks(Fernandes et al., 2022). Clinical studies showed that chronic psychological stress correlated with a variety of peripheral $CD4^+$ T cell abnormalities. Women under chronic caregiving stress showed decreased proportions of both naïve and central memory $CD4^+$ T cell subsets marked by CD45RA in the periphery and increased proportions of effector memory $CD4^+$ T (CD45RA-CD62L-) cells, implicating that chronic stress may accelerate the generation of immunosenescence (Prather et al., 2018). Women with state anxiety symptoms at 12 weeks of gestation were found to be negatively correlated with the proportion of Treg cells, Helios + subpopulation, and $TIM3^+$ subpopulation (Wiley et al., 2024). Therefore, it can be observed that chronic psychological stress can lead to alterations in a wide range of $CD4^+$ T cell subsets.

Cumulative evidence suggests that different subsets of $CD4^+$ T cells correlate with chronic psychological stress and the symptoms of anxiety, suggesting they may play a critical role in the pathophysiology of anxiety disorders. Nevertheless, which $CD4^+$ T cell subsets are altered in anxiety disorder patients and how these abnormal $CD4^+$ T cell subsets are associated with anxiety disorders have not been fully investigated to date. In this study, we first utilized different markers to characterize different $CD4^+$ T-cell subsets including naïve and memory populations (CD27 and CD45RA), activation (CD38, HLA-DR), exhaustion (PD1, TIGIT), effector $CD4^+$ T-cell subsets (CCR4, CCR6, CCR10, CXCR3, CXCR5), $CD4^+$ Tregs (CD25, CD127) (Sahir et al., 2020), as well as senescence phenotype (CD57 and KLRG1) (Rodriguez et al., 2020). We also applied co-stimulatory molecules CD27 and CD28 to characterize the function of activation regulation of $CD4^+$ T cells (Hintzen et al., 1995; Lenschow et al., 1996). Subsequently, we compare them between newly diagnosed adult patients with anxiety disorders and healthy control. Then, we analyzed the associations between differential $CD4^+$ T cell subsets and symptoms' severity of anxiety. Finally, we assessed the mediating effect of differential $CD4^+$T cell subsets on chronic stressful life events to the generation of anxiety by Partial Least Squares-Structural Equation Modeling.

2. Subjects and methods

2.1. Study design and participant enrollment

The study was a case-control study with a matched sample of newly diagnosed anxiety disorder patients and healthy controls. In total, 28 patients with generalized anxiety disorder or panic disorder and 28 matched with age and sex for healthy individuals were enrolled from Peking Union Medical College Hospital between August and December 2022. The inclusion criteria for anxiety disorder patients were as follows: 1) aged between 18 and 45 years, 2) newly diagnosed with generalized anxiety disorder or panic disorder according to the DSM-5 criteria. The exclusion criteria were as follows: 1) use of any antidepressant; 2) Co-existing or previously diagnosed with other severe psychiatric conditions, including schizophrenia, substance abuse, bipolar disorder, and major depression disorder; 3) Currently or previously diagnosed with severe or unstable somatic disease, including, neurological disease, autoimmune diseases, metabolic disease, cardiovascular disease, infectious disease, or cancer; 4) severe brain injuries, or brain lesions; 5) major surgery, vaccination in the past six months; 6) any immunological or metabolic or antibiotic-related medication using in 6 months; 7) the score of 17- item Hamilton Depression Rating scale (HAMD-17) > 17; 8) Peripartum and lactating women. The inclusion criteria for control individuals were as follows: 1) aged between 18 and 45 years; 2) no psychiatric disorders according to DSM-V-RV criteria; 3) no physical severe diseases, and no chronic diseases. The same exclusion criteria were applied to the control group. Written informed consent was obtained from all participants. All human experiments were conducted following protocols approved by the Medical Ethics Committee of Peking Union Medical College Hospital. This study was performed under the ethical standards of the Declaration of Helsinki.

2.2. Clinical assessments

GAD or PD was diagnosed by a structured clinical interview based on the Diagnostic and Statistical Manual of Mental Disorders, fifth edition–Research Version (DSM-V-RV), administered by a psychiatrist and independently confirmed by a trained research assistant.

2.2.1. Severity of anxiety

The severity of anxiety symptoms was measured by the Hamilton Rating Scale for Anxiety (HAMA)(Hamilton, 1959; Thompson, 2015), which is commonly used in clinical settings to assess the level of anxiety experienced by patients, aiding in diagnosis, treatment planning, and monitoring of anxiety disorders. The scale includes 14 items to evaluate psychological anxiety and somatic anxiety, with a total possible score of 56. The HAMA score between 14 and 21 indicates mild anxiety, 21–29 for moderate anxiety, and severe anxiety for the HAMA score more than 29.

2.2.2. Depressive symptoms

Science the depressive symptoms are common for anxiety patients (Kalin, 2020), we utilized the Hamilton Rating Scale for Depression (HAMD-17) (Hamilton, 1960) to assess the severity of depressive symptoms with 17 items over the past week. A total score greater than 17 on the HAMD-17 suggests the possibility of having depression disorder. Previous studies have suggested a higher comorbidity rate between anxiety disorders and depressive disorders (Szuhany and Simon, 2022). Therefore, to exclude the possibility of comorbid depression with anxiety as much as possible, the anxiety patients with a sum score >17 on the HAMD-17 were excluded from our study.

2.2.3. Chronic stressful life events

To evaluate the chronic stressful life events, we used the Life Event Scale (LES) which was a self-report scale containing 48 items (Pérez-Pérez et al., 2018; Y. P. Zheng and Lin, 1994) to evaluate the occurrence and impact of stressful life events over the past year. The version we applied contains 48 items, including job, relationship, health issues, financial difficulties, and other significant life events. The scale aims to assess the stress and emotional impact of these events on the individual. The total score of LES is calculated by summing the weights of each life event. The weights of each life event were evaluated according to the formula: occurrence time of life events × influence duration × the degree of mental impact of life events (Lin et al., 2018). The higher the overall score of LES, the higher the level of stressful mental pressure the individuals perceived.

The HAMA and HAMD were administered and scored by a trained research assistant. The height and weight of all participants were recorded to calculate body mass index (BMI) and analyzed as a continuous variable. Sociodemographic information including age, family history of psychiatric disorders (yes or no), smoking status (ever or never), frequency of physical activity (<1 h/day or ≥1 h/day), and education level (less than 12 years or more than 12 years) were recorded after enrollment in our study.

2.3. Staining and flow cytometry

From all participants, 8 ml of peripheral blood was collected on the second day after diagnosis between 8:00 and 9:00 a.m. in a heparin sodium anticoagulant tube and immediately transported to the laboratory to isolate peripheral blood mononuclear cells (PBMCs). PBMCs were collected using Ficoll density gradient centrifugation per the manufacturer's protocol. Multiparametric flow cytometry panels were developed and validated to characterize $CD4^+$ T-cell subsets. The obtained PBMCs were resuspended in the cell freezing medium (FBS+10% DMSO) and stored in gradient freezing −80 0C for one day before transfer to liquid nitrogen. We thawed the PBMCs per standard protocols, and 1 × 106 PBMCs were prepared to stain with the antibodies of the surface biomarker for each of the three panels. All samples were detected in a batch. Before incubating with antibodies, the red blood cells were lysed with Red Blood Cell Lysis Buffer (Absin). Then, TruStainFcX Fc Receptor Blocking Solution (Biolegend) and True-Stain Monocyte Blocker (Biolegend) were incubated with human PBMCs for 10 min before adding antibodies. The binding buffer was applied for staining, and each sample's final staining reactive volume was 50 μl. The samples were incubated for 25 min at room temperature in the dark, and then the excess reagent was washed out. Finally, 200 μl of 1 X PBS was added to prepare cell suspension for flow cytometry analysis, and FlowJo V9 was used for data analysis. The detailed information on antibodies we applied in different panels is shown in Supplementary Table 1.

2.4. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 28.0. The normality of the distribution of variables was tested using the Kolmogorov–Smirnov test. The differences in variables between the anxiety and control groups were evaluated by an independent-sample *t*-test when the variance was equal, and the distribution was normal. At the same time, the Mann–Whitney *U* test was used to compare the nonparametric variables. In order to control the influence of covariates on the change $CD4^+$ T-cell subsets, we applied ANCOVA analysis to adjust the covariates. Furthermore, we used the False Discovery Rate (FDR) method to correct for the significance of multiple comparisons. FDR-adjusted $P < 0.05$ was set as the statistical significance threshold. To further analyze the relationship between differential $CD4^+$ T-cell subsets and anxiety, we first utilized Person Correlation analysis to evaluate the relationship between differential $CD4^+$ T-cell subsets and anxiety-related variables, including LES, HAMA, HAMD-17, and BMI. Then, multiple linear regression models were constructed to evaluate the effect of $CD4^+$ T-cell subsets and other factors on the severity of anxiety. The overall severity of anxiety was assessed by HAMA, and the severity of psychological anxiety and somatic anxiety was evaluated by the subscale of HAMA. Stepwise multiple linear regression was chosen because the stepwise method combines advantages of forward and backward selection. After introducing each new independent variable, the method re-evaluates the previously included variables to determine their continued value in the equation. Based on this evaluation, the model alternates between introducing and removing variables until no new variables can be added or removed. This approach helps mitigate the effects of multicollinearity in multivariate linear regression analysis (Malek et al., 2007). In the regression model, we included all sociodemographic and physical characteristics (educational levels, family history of psychiatric disorders, BMI, as well as total LES scores as independent variables) we obtained to assess the influence of these potentially confounding variables on $CD4^+$ T-cell subsets on the severity of anxiety.

Finally, Smart Partial Least Squares (SmartPLS)-SEM path analysis (Riou et al., 2016) was applied to evaluate our hypothesized mediating effect and interactive effect between the immune system and stressful life events on the generation of anxiety. To rule out the potential influence of BMI on the generation of anxiety, BMI was involved in our model. Therefore, the HAMA score was constructed as latent, representing anxiety. Total LES score and BMI were constructed as latent, representing stressful life events and metabolic conditions with a single indicator. According to previous studies, psychological stress and BMI influenced the memory phenotype of $CD4^+$T cell subsets and regulatory $CD4^+$T cell subsets. Thus, two latent vectors, which represent immune response (memory phenotype) and immune regulation (regulatory phenotype) were built. Complete SmartPLS analysis using 5000 bootstrap samples was performed when the inner/outer models complied with specific quality data: 1) the overall fit of the pathway model is adequate with standardized root mean square residual (SRMR) < 0.08; 2) the outer model latent vector loadings are >0.6 at $p < 0.001$; and 3) the latent vectors show an accurate construct validity as indicated by an average variance extracted (AVE) > 0.5, Cronbach's alpha >0.7, and composite reliability >0.7. Consequently, we conducted a complete PLS path analysis on 5000 bootstrap samples and computed path coefficients (with p-value), outer model loadings, and specific indirect and total effects.

3. Results

3.1. Baseline characteristics

Fifty-three newly diagnosed patients who visited the Department of Psychiatry outpatient clinic at Peking Union Medical College Hospital were screened. Sixteen of them were excluded because ten patients with generalized anxiety disorder met the comorbidity criteria for depression disorder after screening, and the other six generalized anxiety disorder patients met the criteria for previous depressive disorder. Other nine patients were excluded due to HAMD-17 > 17. Finally, a total of 56

participants were enrolled, including 28 newly diagnosed drug naïve anxiety disorders (AD) patients (10 with panic disorder and 18 with generalized anxiety disorder) and 28 healthy controls (HC). There were no significant differences in age, sex, smoking status, or physical activity between the AD and HC groups (Table 1). All participants in the HC group had received more than 12 years of education, while 7.14% of patients (2/28) in the AD group had received less than 12 years of education. BMI, HAMA scores, HAMA-psychological anxiety scores, HAMA-somatic anxiety scores, HAMD-17 scores, and total LES scores were significantly different between the two groups ($P < 0.05$). None had a family history of psychiatric disorders in both the AD and HC groups.

3.2. Differential $CD4^+$ T-cell subset in anxiety disorders

To determine which $CD4^+$ T-cell subsets were altered in anxiety disorder patients, the multiparametric flow cytometry was applied to identify 44 different CD4+T cell subsets (Supplementary Table 2); the gating strategy of each subpopulation is shown in Supplementary Fig. 1. Among the 44 CD4+T cell subsets we identified, 10 showed significant differences between the two groups (Table 2). Th and Treg cell subsets of CD27CD45RA and CD27CD28 significantly differed between the two groups. The numbers of $CD27^+CD45RA^-$ Th, $CD27^+CD28^-$Th, $CD27^-CD28^+$ Th, and $CD27^-CD28^-$ Th cells were significantly higher, while $CD27^+CD45RA^+$Th and $CD27^+CD28^+$ Th cells were markedly lower in the AD group. It was also apparent that the proportion of $CD27^+CD28^+$ Tregs was lower in the AD group than in the HC group, while the frequency of $CD27^+CD28^-$ Tregs was significantly higher in the AD group. The subsets of $CD27^+CD45RA^-$Tregs and $CD27^-CD45RA^-$Tregs showed an opposite trend in the Anxiety disorders group. All of these outcomes showed that subsets of naïve and memory subsets of Th and Treg cells were dysregulated in anxiety disorders. Due to the significant statistical difference in BMI between the two groups of subjects, we included BMI as a covariate and used ANCOVA to analyze the effect of BMI on the frequency of distinct $CD4^+$ T-cell subsets between the two groups. The results indicate that, except for $CD27^-$CD45RA-Tregs, other differential $CD4^+$ T cell subsets between the two groups are not affected by BMI (Table 3, Supplementary Table 3). After FDR correction for multiple comparisons, the differences in the proportions of $CD27^-$CD28-Th, $CD27^-$CD45RA-Tregs, and $CD27^-$CD28-Tregs between the two groups are no longer significant (Fig. 1). The population of activation, exhaustion, and senescence of CD4+T subsets have no significant difference between anxiety disorders and HC. Furthermore, the effector subsets, including Th1, Th2, Th17, and Tfh ($CXCR5^+$) cells, showed no difference between the two groups.

Table 1
Clinical characteristics of anxiety disorder patients and matched healthy controls.

	Anxiety patients	Healthy controls	P value
	N = 28	N = 28	
Sex			0.567
Female	20	18	
	71.4%	64.3%	
Male	8	10	
	28.6%	35.7%	
Family history of psychiatric disorders			–
No	28	28	
	100%	100%	
Yes	0	0	
	0.00%	0.00%	
Smoking status			0.16
Never	24	27	
	87.5%	96.4%	
Ever	4	1	
	14.3%	3.6%	
Physical activity			0.789
<1 h/day	15	14	
	53.6%	50.0%	
≥1 h/day	13	14	
	46.4%	50.0%	
Educational level			0.149
<12 years	2	0	
	7.14%	0.00%	
≥12 years	26	28	
	92.86%	100%	
Age, years (median)	34.50 (30.50, 39.75)	33.50 (27.00, 36.00)	0.249
BMI, kg/m^2 (median)	21.79 (19.98, 23.71)	18.93 (17.19, 21.18)	<0.001[a]
HAMA			
Total HAMA score (median)	26.00 (24.25, 28.75)	7.00 (6.25, 9.00)	<0.001[a]
Psychological anxiety (median)	13.00 (11.75, 15.00)	3.00 (2.00, 4.00)	<0.001[a]
Somatic anxiety (median)	13.00 (11.75, 15.25)	4.00 (3.00, 5.00)	<0.001[a]
HAMD-17 score (median)	9.50 (8.00, 13.75)	7.00 (5.00, 8.00)	<0.001[a]
LES total score (median)	63.50 (48.0, 169.25)	20.50 (13.25, 30.75)	<0.001[a]

BMI: body mass index, HAMA: Hamilton Anxiety Rating Scale, HAMD: Hamilton Depression Rating Scale, LES: Life Event Scale.
*Values are presented as the number (%) and the results of chi-square tests.
[a] Values are presented as the median (25th and 75th percentiles) and analyzed by the Mann–Whitney *U* test.

Table 2
The differential subsets of $CD4^+$ T cells in anxiety disorder patients and healthy controls.

CD4+T-cell subsets %	Anxiety patients	Healthy controls	P value	FDR-adjusted
	N = 28	N = 28		
T cells[b]	75.3 (70.3, 81.2)	64.1 (58.1, 75.4)	0.005	0.013[c]
CD4+T cells[a]	55.22 ± 2.31	48.98 ± 1.75	0.018	0.040[c]
$CD27^+CD45RA^+$ Th cells[b]	34.9 (21.1, 43.9)	48.2 (41.4, 54.0)	<0.001	0.0007[c]
$CD27^+CD45RA^-$ Th cells[a]	41.34 ± 2.64	27.41 ± 1.63	0.009	0.022[c]
$CD27^+CD28^+$ Th cells[b]	66.0 (58.3, 73.4)	83.3 (77.8, 88.1)	<0.001	<0.001[c]
$CD27^+CD28^-$ Th cells[b]	1.15 (0.670, 2.36)	0.60 (0.270, 1.05)	0.005	0.013[c]
$CD27^-CD28^+$ Th cells[b]	27.1 (20.1, 36.6)	14.2 (9.49, 17.2)	<0.001	<0.001[c]
$CD27^-CD28^-$ Th cells[b]	4.04 (1.67, 8.28)	1.95 (1.15, 2.94)	0.017	0.059[c]
$CD27^+CD45RA^-$ Tregs[a]	58.34 ± 2.97	46.94 ± 2.10	0.006	0.016[c]
$CD27^-CD45RA^-$ Tregs[a]	16.86 ± 2.18	23.66 ± 1.66	0.04	0.075
$CD27^+CD28^+$ Tregs[b]	35.1 (23.9, 49.1)	78.9 (74.3, 80.8)	<0.001	<0.001[c]
$CD27^+CD28^-$ Tregs[b]	35.5 (9.63, 48.4)	0.245 (0.105, 0.350)	<0.001	<0.001[c]
$CD27^-CD28^-$ Tregs[b]	5.64 (2.73, 9.67)	1.74 (0.675, 7.05)	0.046	0.085

[a] Values are presented as the mean ± SD and analyzed by the independent-sample *t*-test.
[b] Values are presented as the median (25th and 75th percentiles) and analyzed by the Mann–Whitney *U* test.
[c] FDR-adjusted P values < 0.05 were considered statistically significant.

3.3. Correlations analysis between differential $CD4^+$ T-cell subsets and clinical variables

We performed person correlation analysis to detect the relationship between differential $CD4^+$ T-cell subsets and anxiety-related variables including total LES score, HAMA, HAMD-17 as well as the potential confounding variable of BMI (Fig. 2). Total LES score was negatively correlated with $CD27^+CD45RA^+$ Th ($r = -0.44$, $P = 0.002$),

Table 3
The Differential Subsets of $CD4^+$ T Cells in Anxiety Disorder Patients and Healthy Controls adjusted by BMI.

CD4+T-cell subsets	Index	df	F	PR(>F)[a]
T cells	C(Group)	1	11.903	0.001
	BMI	1	2.939	0.092
	Residual	53		
CD4+T cells	C(Group)	1	4.083	0.048
	BMI	1	0.201	0.655
	Residual	53		
$CD27^+CD45RA$ + Th cells	C(Group)	1	12.128	0.001
	BMI	1	0.315	0.577
	Residual	53		
$CD27^+CD45RA$- Th cells	C(Group)	1	10.760	0.002
	BMI	1	1.428	0.237
	Residual	53		
$CD27^+CD28^+$ Th cells	C(Group)	1	26.160	<0.001
	BMI	1	0.033	0.856
	Residual	53		
$CD27^+CD28^-$ Th cells	C(Group)	1	4.553	0.038
	BMI	1	0.043	0.856
	Residual	53		
$CD27^-CD28^+$ Th cells	C(Group)	1	39.678	<0.001
	BMI	1	0.169	0.683
	Residual	53		
$CD27^-CD28^-$ Th cells	C(Group)	1	4.213	0.036
	BMI	1	0.758	0.387
	Residual	53		
$CD27^+CD45RA$- Tregs	C(Group)	1	8.892	0.008
	BMI	1	5.629	0.066
	Residual	53		
$CD27^-CD45RA$- Tregs	C(Group)	1	2.779	0.101
	BMI	1	1.527	0.222
	Residual	53		
$CD27^+CD28^+$ Tregs	C(Group)	1	94.451	<0.001
	BMI	1	0.011	0.917
	Residual	53		
$CD27^+CD28^-$ Tregs	C(Group)	1	48.092	<0.001
	BMI	1	0.013	0.910
	Residual	53		
$CD27^-CD28^-$ Tregs	C(Group)	1	5.371	0.024
	BMI	1	0.586	0.447
	Residual	53		

Abbreviation: BMI: Body Mass Index.
[a] : P-value.

$CD27^+CD28^+$ Th ($r = -0.46$, $P = 0.001$), as well as $CD27^+CD28^+$ Tregs ($r = -0.38$, $P = 0.007$) and positively with $CD27^+CD28^-$ Th ($r = 0.32$, $P = 0.023$), $CD27^+CD45RA^-$ Tregs ($r = 0.33$, $P = 0.014$), as well as $CD27^+CD28^-$ Tregs ($r = 0.31$, $P = 0.037$).

HAMA score showed significant correlation with all eight differential CD4+T cell subsets (Fig. 3), which were positively correlated with $CD27^+CD45RA^-$ Th ($r = 0.52$, $P < 0.001$), $CD27^+CD28^-$ Th ($r = 0.28$, $P = 0.046$), $CD27^-CD28^+$ Th ($r = 0.63$, $P < 0.001$), $CD27^+CD45RA^-$ Tregs ($r = 0.389$, $P = 0.006$), $CD27^+CD28^-$ Tregs ($r = 0.68$, $P < 0.001$), and negatively with $CD27^+CD45RA^+$ Th ($r = -0.50$, $P < 0.001$), $CD27^+CD28^+$ Th ($r = -0.58$, $P < 0.001$), as well as $CD27^+CD28^+$ Tregs ($r = -0.76$, $P < 0.001$).

We also observed a significant correlation of three subsets with BMI, including $CD27^+CD45RA^-$ Th cells ($r = 0.33$, $P = 0.015$), $CD27^+CD45RA^-$ Tregs ($r = 0.40$, $P = 0.005$), and $CD27^+CD28^+$ Tregs (-0.34, $P = 0.03$). $CD27^+CD28^+$ Tregs was the only subset that correlated with LES, BMI, HAMA, and HAMD-17. Moreover, according to correlation analysis, there are significant strong correlations between the proportion of $CD27^+CD45RA^+$ Th and $CD27^+CD28^+$ Th cells, $CD27^+CD28^+$ Th and $CD27^-CD28^+$ Th cells, $CD27^+CD45RA^-$ cells and $CD27^+CD45RA^-$ Treg cells, as well as $CD27^+CD28^+$ Treg and $CD27^+CD28^-$ Treg cells, suggesting there is collinearity among these populations.

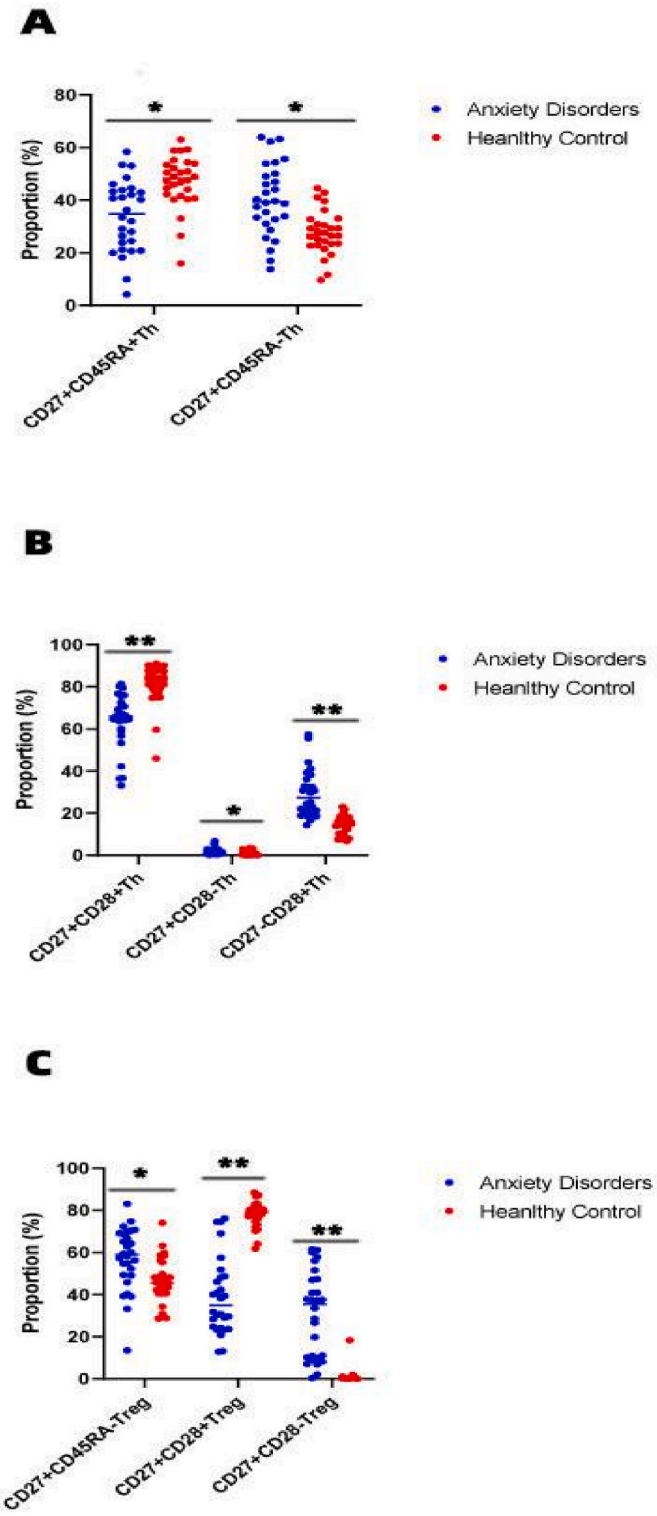

Fig. 1. The dot-plot of differential CD4+T cell subsets between anxiety disorders and healthy control with FDR-adjusted $P < 0.05$
A, the proportion of naïve and central memory CD4+T subsets ($CD27^+CD45RA$ + Th and $CD27^+CD45RA$-Th); B, the proportion of $CD27^+CD28^+$ Th, $CD27^+CD28$-Th and $CD27^-CD28$+Th subsets; C, the proportion of $CD27^+CD45RA$-Treg, $CD27^+CD28^+$ Treg, as well as $CD27^+CD28^-$ Treg cell subsets. (* represents FDR-adjusted $P < 0.05$, ** represents FDR-adjusted $P < 0.001$).

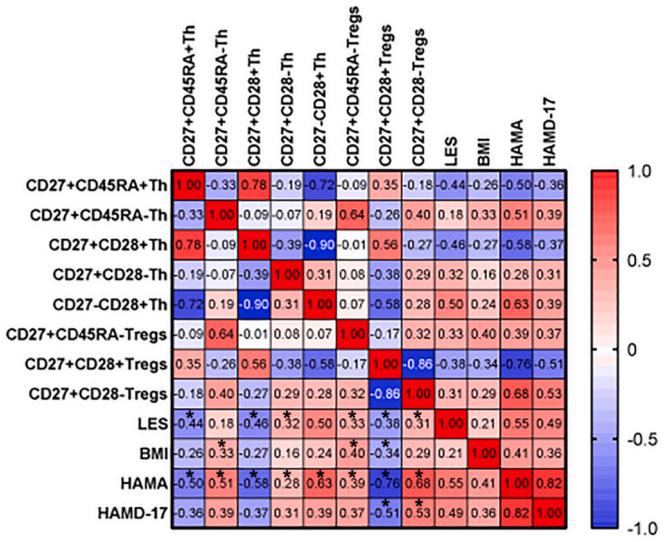

Fig. 2. Heatmap of correlation analysis between the significant difference of CD4+T cell subsets and anxiety-related variables
Abbreviation: BMI: Body Mass Index, HAMA: Hamilton Anxiety Rating Scale, HAMD: Hamilton Depression Rating Scale, LES: Life Event Scale.
The colors represent correlations, where red indicates a positive correlation, and blue indicates a negative correlation. * mean significant correlation between $CD4^+$ T-cell subsets and LES, BMI, HAMA, and HAMD-17.

3.4. Association of differential $CD4^+$ T-cell subsets with anxiety

To further explore the association of differential $CD4^+$ T cells and the severity of anxiety, the stepwise multivariate linear regression model was applied because, according to the results of our correlation analysis, there is collinearity between different $CD4^+$ T cell subsets. To eliminate the influence of collinearity, we employed stepwise multiple regression analysis to analyze the total HAMA and subscale scores as dependent variables and the immune subsets and other clinical variables as explanatory variables (Table 4). The proportion of $CD27^+CD45RA^-$ Th cells and total LES showed a positive association with total HAMA scores [beta:0.325, $P < 0.001$, beta:0.270, $P = 0.001$], while the $CD27^+CD28^+$ Tregs was negatively associated with total HAMA scores [beta: −0.568, $P < 0.001$]. 72.4% of the variance in total HAMA scores could be explained by the regression on $CD27^+CD28^+$ Tregs, $CD27^+CD45RA^-$ Th cells, and total LES (model 2). For the HAMA-psychological anxiety, 64.7% of the variance in HAMA-psychological anxiety could be explained by the change of $CD27^+CD28^+$ Tregs, $CD27^+CD45RA^-$ Th cells, and total LES (model 4). Model 7 showed that 66.1% of the variance in HAMA-somatic anxiety could be explained by $CD27^+CD28^+$ Tregs, $CD27^+CD28^+$ Th cells, $CD27^+CD45RA^-$ Th cells, as well as total LES.

Since depression is one common comorbid symptom in anxiety disorder patients(Kalin, 2020), we also explored the association of differential $CD4^+$ T-cell subsets with depression symptoms in anxiety patients. Only 28.2% of the variance in HAMD-17 score could be explained by the change of $CD27^+CD28^-$Tregs and total LES score (Table 5), indicating that the $CD4^+$ T-cell subset contributing to the variance of depression symptom in anxiety patients was different from anxiety.

3.5. Results of partial least squares (PLS) analysis

Furthermore, to explain how the $CD4^+$ T-related immune system and long-term psychological stress impact anxiety, we utilized the SmartPLS to examine our hypothesized pathway model on the entire sample. Because $CD27^+CD45RA^-$ Th/Treg subsets were considered as memory subsets of T cells and $CD27^+CD28^+$ Th/Treg subsets were believed as naïve population(Okada et al., 2008; Sallusto et al., 2004), we extracted $CD27^+CD45RA^-$ Th/Treg subsets as the latent vector to reflect the status of immune response and $CD27^+CD28^+$ Th/Treg subsets as the other latent vector to represent the status of immune regulation. We also added BMI as an indicator to evaluate the interaction of metabolic status, immune system, and anxiety.

Fig. 4A showed that the immune response, LES, and BMI could explain 44.8% of the anxiety variance. However, there were no significant specific indirection effects of both LES and BMI on the immune response to anxiety ($t = 1.163$, $P = 0.245$; $t = 1.845$, $P = 0.065$), indicating that immune response does not mediate the effects of LES or BMI on anxiety. This model showed a significant impact of LES on anxiety, the effects of immune response on anxiety, as well as BMI on anxiety. The construction of the latent immune response is good with Cronbach $\alpha = 0.788$, AVE = 0.825, rho_a = 0.789, and rho_c = 0.904. The overall fit of the model was adequate with the SRMR = 0.056.

Fig. 4B showed that immune regulation, LES, and BMI explained 64.1% of the variance in anxiety. There was a significant specific indirect effect from LES to immune regulation on anxiety, demonstrating that immune regulation mediates the effect of LES on anxiety ($t = 2.871$, $P = 0.004$). The pathway from BMI to anxiety was not statistically significant with $P > 0.05$ ($P = 0.083$), while there was no significant total indirect effect of BMI on anxiety ($t = 1.862$, $P = 0.063$). The construction of the latent immune regulation is good with Cronbach $\alpha = 0.704$, AVE = 0.770, rho_a = 0.717, and rho_c = 0.870. The overall fit of the model was adequate with the SRMR = 0.067.

Since long-term psychological stress has a bidirectional influence on the immune system(Biltz et al., 2022), we postulated that the change in the immune system may interact with chronic psychological stress on anxiety. Subsequently, we reconstructed the model to test the interaction effects of $CD4^+$ T-cell-related immune system to chronic stress on anxiety. Fig. 5 displayed the adjusted model. 80.2% of the variance of anxiety can be directly explained by the immune response ($t = 2.943$, $P = 0.003$), immune regulation ($t = 3.171$, $P = 0.002$), and LES ($t = 4.234$, $P < 0.001$), however, BMI do not have a total effect on anxiety ($t = 0.143$, $P = 0.100$). BMI and LES could partially explain 26.5% of the variance of immune regulation and 20.2% of the variance of immune response. Furthermore, there was a significant specific indirect effect of LES on immune regulation on anxiety ($t = 2.212$, $P = 0.027$), which is consistent with the finding from the above model, indicating that $CD27^+CD28^+$ Th/Treg cells inversely mediate the effect of LES on anxiety. Moreover, there were statistically significant effects of interaction between immune regulation and LES on anxiety ($t = 2.161$, $P = 0.031$). The overall fit of the adjusted model was adequate with SRMR = 0.074 and good construct reliability of two immune-related latent vectors with Cronbach $\alpha > 0.704$, AVE >0.770, rho_a >0.717, and rho_c > 0.870, vectors loadings were all >0.850.

4. Discussion

In this study, we analyzed the differentiation phenotype, exhaustion phenotype, senescence phenotype, and functional phenotype of $CD4^+$ T cells using a multi-channel flow cytometry detection method. Upon activation by antigen stimulation, co-stimulatory molecule activation, and cytokine stimulation, naïve $CD4^+$ T cells undergo proliferation and differentiation to form different effector cell subsets and memory cell pools, thereby exerting effector functions and maintaining immune homeostasis(Kumar et al., 2018). CD27 and CD28, as co-stimulatory molecules, play essential roles in maintaining the survival of $CD4^+$ T cells, inducing proliferation, aging, and regulating immune homeostasis (Kovaiou et al., 2005). Previous studies have shown that chronic psychological stress can lead to exhaustion and senescence phenotypes of $CD4^+$ T cells, and exhaustion and senescence are closely related to T cell function and homeostasis(Gouin et al., 2008). Therefore, characterizing the differentiation phenotype, exhaustion phenotype, senescence phenotype, and functional phenotype can comprehensively reflect the function and homeostasis of $CD4^+$ T cells.

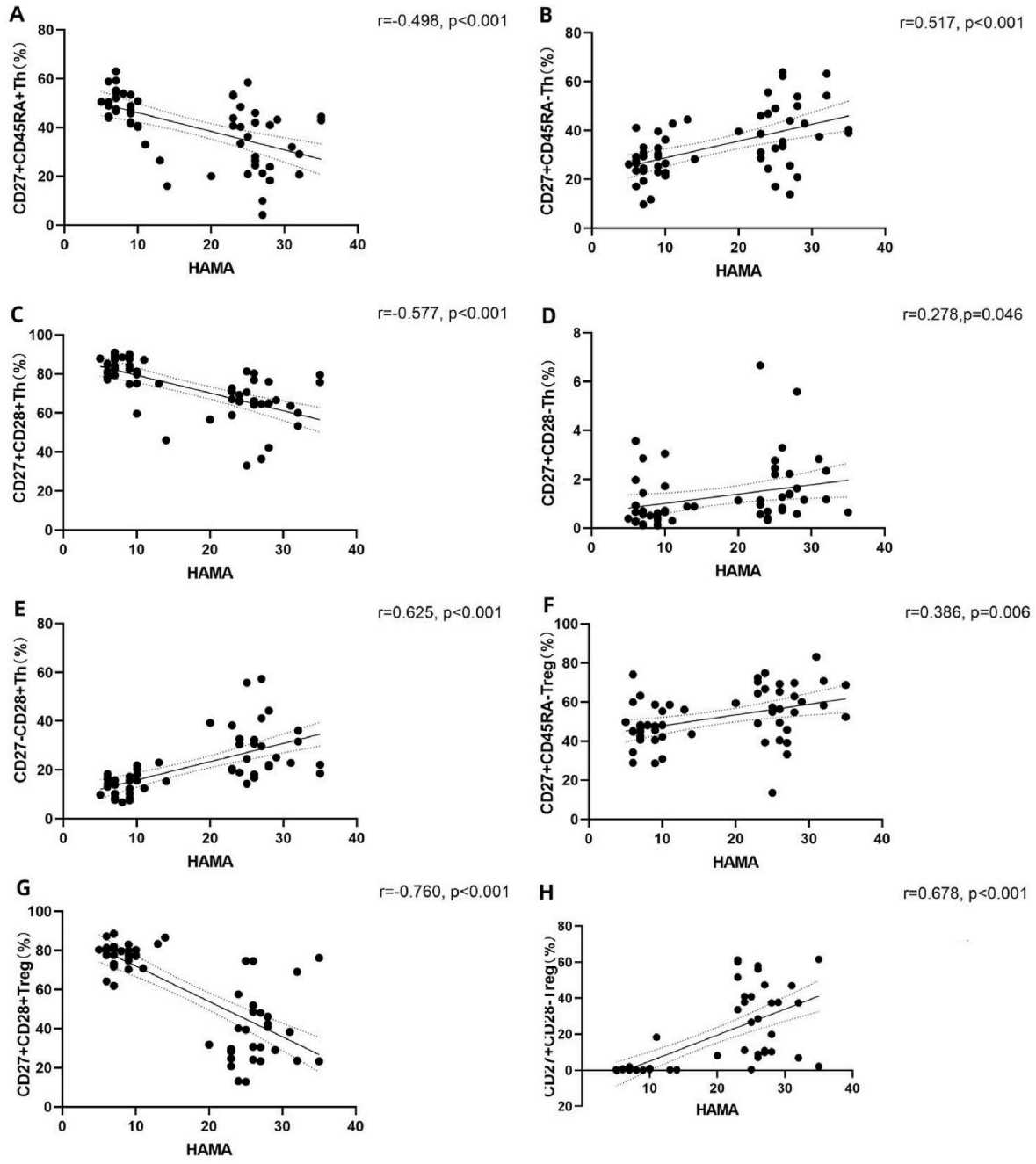

Fig. 3. The correlation XY plot between the proportions of differential $CD4^+$ T cell subsets and HAMA scores
The correlation between HAMA and proportion of $CD27^+CD45RA$ + Th (A), $CD27^+CD45RA$-Th (B), $CD27^+CD28$+Th (C), $CD27^+CD28$-Th (D), $CD27^-CD28$+Th (E), $CD27^+CD45RA$- Tregs (F), $CD27^+CD28^+$ Tregs (G), and $CD27^+CD28^-$ Tregs (H).

Our results indicate that compared to healthy controls, patients with anxiety disorders without treatment exhibit a significant decrease in the proportion of $CD27^+CD28^+$ Th and Treg cells. In contrast, the proportion of central memory Th and Treg cells ($CD27^+CD45RA^-$) significantly increases. There have been few studies on the distribution of different $CD4^+$ T cell subsets in anxiety disorders, and only a few studies have explored the association between $CD4^+$ T cells and anxiety levels. Gu et al. characterized the peripheral $CD4^+$ T cell subsets of systemic lupus erythematosus patients with or without anxiety symptoms using a hospital Anxiety and Depression Scale score $\geq$ eight as the criterion for anxiety status. The results showed that systemic lupus erythematosus patients with anxiety symptoms had a decreased proportion of $CD27^+CD28^+$ Th and Treg cells in peripheral $CD4^+$ T cells. In contrast, the proportion of effector memory $CD4^+$ T cell subsets ($CD27^-CD45RA^-$ Th/Treg) increased (Gu et al., 2021), suggesting that changes in the proportion of $CD27^+CD28^+$ Th/Treg cell subsets in peripheral $CD4^+$ T cells are more consistent with the association with anxiety in different backgrounds, indicating that $CD27^+CD28^+$ Th/Treg cell subsets in peripheral $CD4^+$ T cells may play a vital role in the occurrence of anxiety.

There is currently no research on the changes of different memory $CD4^+$ T cell subsets in patients with anxiety disorders. In contrast to the outcomes from Gu et al., we found the frequency of central memory subsets of $CD4^+$ T cells increased with statistical significance in anxiety disorder patients. Due to the significant increase in the proportion of effector memory $CD4^+$ T cell subsets in systemic lupus erythematosus patients and the lower proportion of central memory $CD4^+$ T cells

Table 4
Multivariate linear regression for association of differential $CD4^+$ T-Cell subsets and anxiety.

Dependent Variables	Independent Variables	B	t	Sig.	F model	df	P	R^2	FDR Corrected P
Total HAMA	Model 1				51.604	2	<0.001	0.665	<0.001
	$CD27^+CD28+$Tregs	−0.662	−7.908	<0.001					
	$CD27^+CD45RA$-Th	0.352	4.204	<0.001					
	Model 2				45.557	3	<0.001	0.724	<0.001
	$CD27^+CD28+$Tregs	−0.568	−7.028	<0.001					
	$CD27^+CD45RA$-Th	0.325	4.255	<0.001					
	Total LES	0.270	3.384	0.001					
HAMA psychological anxiety	Model 3				41.288	2	<0.001	0.617	<0.001
	$CD27^+CD28+$Tregs	−0.594	−6.483	<0.001					
	$CD27^+CD45RA$-Th	0.383	4.180	<0.001					
	Model 4				31.576	3	<0.001	0.647	<0.001
	$CD27^+CD28+$Tregs	−0.526	−5.671	<0.001					
	$CD27^+CD45RA$-Th	0.356	4.011	<0.001					
	Total LES	0.206	2.258	0.029					
	Model 5				26.716	4	<0.001	0.673	<0.001
	$CD27^+CD28+$Tregs	−0.489	−5.376	<0.001					
	$CD27^+CD45RA$-Th	0.300	3.356	0.002					
	Total LES	0.194	2.204	0.033					
	BMI	0.193	2.166	0.036					
HAMA Somatic anxiety	Model 6				30.045	3	<0.001	0.635	<0.001
	$CD27^+CD28+$Tregs	−0.566	−5.999	<0.001					
	Total LES	0.280	3.019	0.004					
	$CD27^+CD45RA$-Th	0.213	2.358	0.023					
	Model 7				25.324	4	<0.001	0.661	<0.001
	$CD27^+CD28+$Tregs	−0.458	−4.385	<0.001					
	Total LES	0.210	2.211	0.032					
	$CD27^+CD45RA$-Th	0.245	2.776	0.008					
	$CD27^+CD28+$Th	−0.224	−2.117	0.040					

Abbreviation: BMI: Body Mass Index, HAMA: Hamilton Anxiety Rating Scale, LES: Life Event Scale.

Table 5
Association of differential $CD4^+$ T-cell subsets and HAMD.

Dependent Variables	Independent Variables	B	t	Sig	F Model	df	P	R^2	FDR Corrected P
HAMD-17	Model 1				14.316	1	<0.001	0.207	0.0013
	$CD27^+CD28$-Tregs	0.472	3.784	<0.001					
	Model 2				11.021	2	<0.001	0.282	<0.001
	$CD27^+CD28$-Tregs	0.382	3.082	0.003					
	Total LES	0.309	2.496	0.016					

Abbreviation: HAMD: Hamilton Depression Rating Scale, LES: Life Event Scale.

(Fritsch et al., 2006), this may lead to a more significant increase in effector memory $CD4^+$ T cell subsets in systemic lupus erythematosus patients with anxiety symptoms. Currently, the main hypotheses regarding the development pathways of memory cells include the linear model hypothesis, the asymmetric model hypothesis, the self-renewal model hypothesis, and the simultaneous occurrence hypothesis (Ahmed et al., 2009). According to the asymmetric model and self-renewal hypothesis, naïve T cells can form central memory cells through self-renewal and antigen exposure (Pepper and Jenkins, 2011). Our study found that patients with anxiety disorders exhibited a significantly decreased proportion of naïve subset, which may contribute to the increase of central memory $CD4^+$ T cell subsets by asymmetric or self-renewal pathway.

The association between changes in the proportion of different $CD4^+$ T cell subsets and chronic psychological stress varies among different populations. In women exposed to long-term caregiving stress, there is an increase in the proportion of effector memory $CD4^+$ T cells and a decrease in the proportion of central memory $CD4^+$ T cells (Prather et al., 2018). Studies in populations over 50 years old have found that chronic stress decreases the proportion of naïve $CD4^+$ T cells (Klopack et al., 2022). Consistent with previous research, our study found a negative correlation between high levels of stressful life events exposure and the proportion of naïve $CD4^+$ T cell subsets ($CD27^+CD45RA^+$ Th, $CD27^+CD28^+$ Th/Treg). In addition, the level of stressful life events was also found to be positively correlated with the central memory $CD4^+$ T cell subset ($CD27^+CD45RA^-$ Th/Treg). In this study, we also observed a positive correlation between BMI and the frequency of central memory $CD4^+$ T cells and a negative correlation with $CD27^+CD28^+$ Treg cells. Consistent with the previous findings on metabolic disorders and Tregs that higher BMI values correlate with low suppressive activity of Tregs (Cohen et al., 2019; Zhu et al., 2019), however, the influence of BMI on specific Treg subsets has not been widely studied. It is worth noting that changes in the frequencies of specific $CD4^+$ T cell subsets may also be influenced by altering other subsets under the same parent gate. Such as the proportion changes of $CD27^+CD28^+$ Treg and $CD27^+CD28^-$ Treg subsets, as well as $CD27^+CD28^+$ Th and $CD27^-CD28^+$ Th subsets, exhibiting significant strong correlations between them.

Stepwise multiple linear regression analysis showed that $CD27^+CD45RA^-$ Th cells, $CD27^+CD28^+$ Tregs, and LES dependently associate with the severity of anxiety after controlling the influence of collinearity. Furthermore, PLS-SEM analysis was applied and found a specific indirect effect from chronic life events stress to immune regulation to anxiety, validating the mediating impact of $CD27^+CD28^+$ Th/Tregs on the total LES to anxiety. Moreover, we found the interaction effect of $CD27^+CD28^+$Th/Tregs with total LES on anxiety. It is worth noting that the immune response reflected by the central memory subsets of $CD27^+CD45RA^-$ Th cells and $CD27^+CD45RA^-$ Tregs shows a significant positive effect on anxiety. However, they do not mediate the impact of LES on anxiety. The result from PLS-SEM suggested that a high level of $CD27^+CD28^+$ Th/Tregs proportion may be the protective factor in generating anxiety. A recent study on generalized anxiety disorder by utilizing the mendelian randomization revealed that $CD4^+CD28^+$ T cell

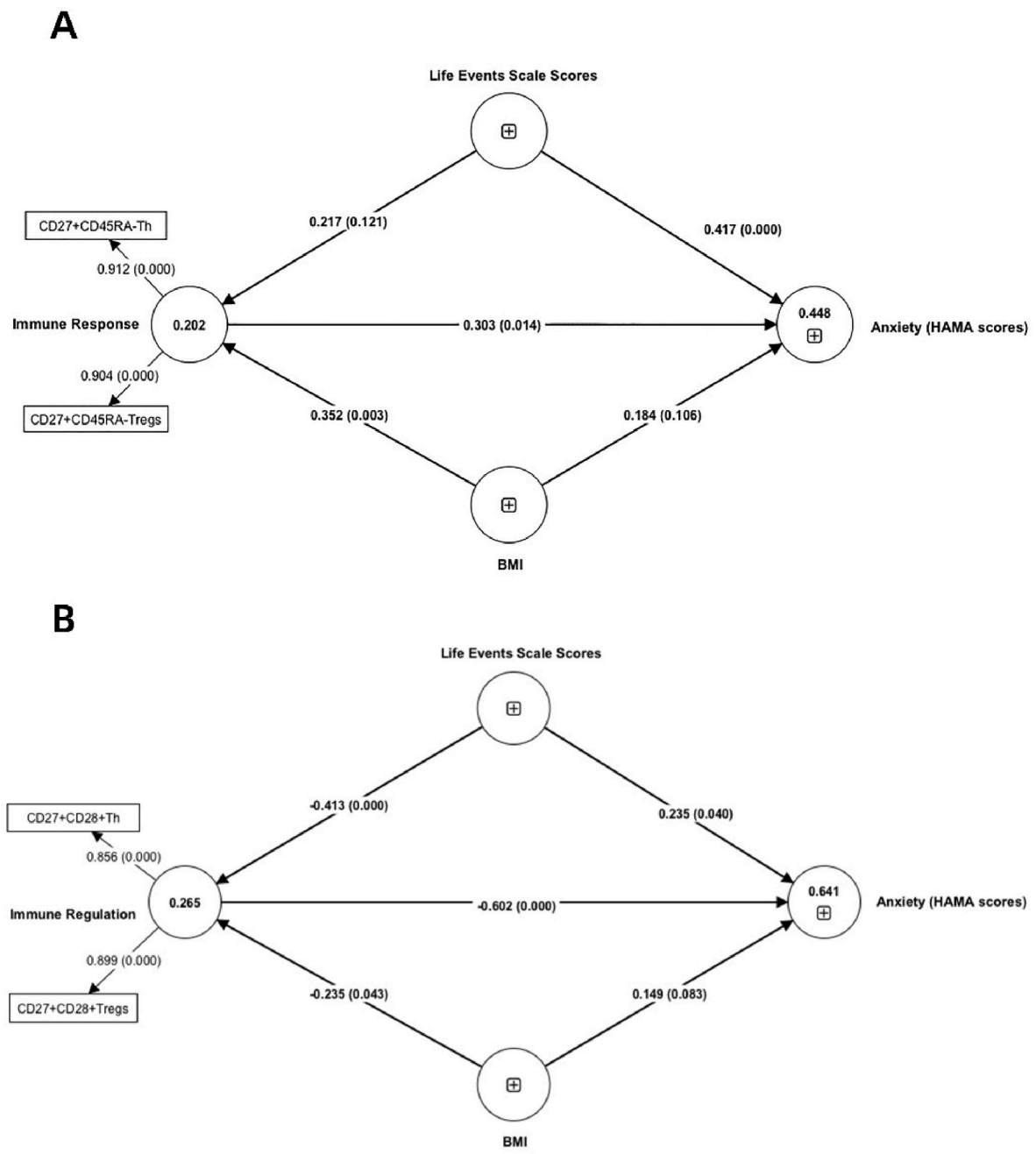

Fig. 4. Results of Partial Least Squares (PLS)-SEM analysis with the Anxiety (total HAMA score) as output variable, the mediating effect model.
The immune response as indicated by a latent vector extracted from $CD27^+CD45RA^-$ Th and $CD27^+CD45RA^-$ Treg cells, the immune regulation as indicated by the latent vector extracted from $CD27^+CD28^+$ Tregs and $CD27^+CD28^+$ Th cells. The path coefficients or latent vector loadings were shown with accompanying p-values. The mediating effect of Immune Response to stressful life events on anxiety (A); The mediating effect of Immune Regulation to stressful life events on anxiety (B). BMI: Body Mass Index; HAMA: Hamilton anxiety scale.

is associated with the risk of generalized anxiety disorder (Ma et al., 2023), confirming the protective role of $CD4^+CD28^+$ T cell subsets in anxiety.

So far, the studies focusing on the mechanism of different $CD4^+$ T cell subsets on the generation of anxiety are still limited. The homeostasis of $CD4^+$ T cells is associated with a range of brain functions, including learning, memory, and anxiety-like behaviors (Filiano et al., 2016). Previous studies have found that the activation of peripheral $CD4^+$ T cells is critical for their entry into the brain (Kunkl et al., 2020; Nishihara et al., 2020) and responsible for the development and maturation of microglia and synapse pruning (Pasciuto et al., 2020). Abnormal microglial maturation and synapse pruning may lead to anxiety-like behavior (McKim et al., 2018; Z. H. Zheng et al., 2021), which links the role of peripheral $CD4^+$ T cells in the functional regulation of the brain. In our study, the proportion of $CD27^+CD45RA^-$ Th cells independently associated with the severity of anxiety positively, and we postulated that this subset may directly contribute to anxiety by their inflammatory role in the brain. It has been investigated that central memory $CD4^+$ T cells are dominantly abundant in cerebrospinal fluid (de Graaf et al., 2011), which can be activated rapidly to execute inflammatory processes or autoimmune responses by encountering the specific antigen (Engelhardt and Ransohoff, 2005). Studies on multiple sclerosis showed that central memory $CD4^+$ T cells with Th17 phenotype correlated with the severity of the disease and strongly resembled the

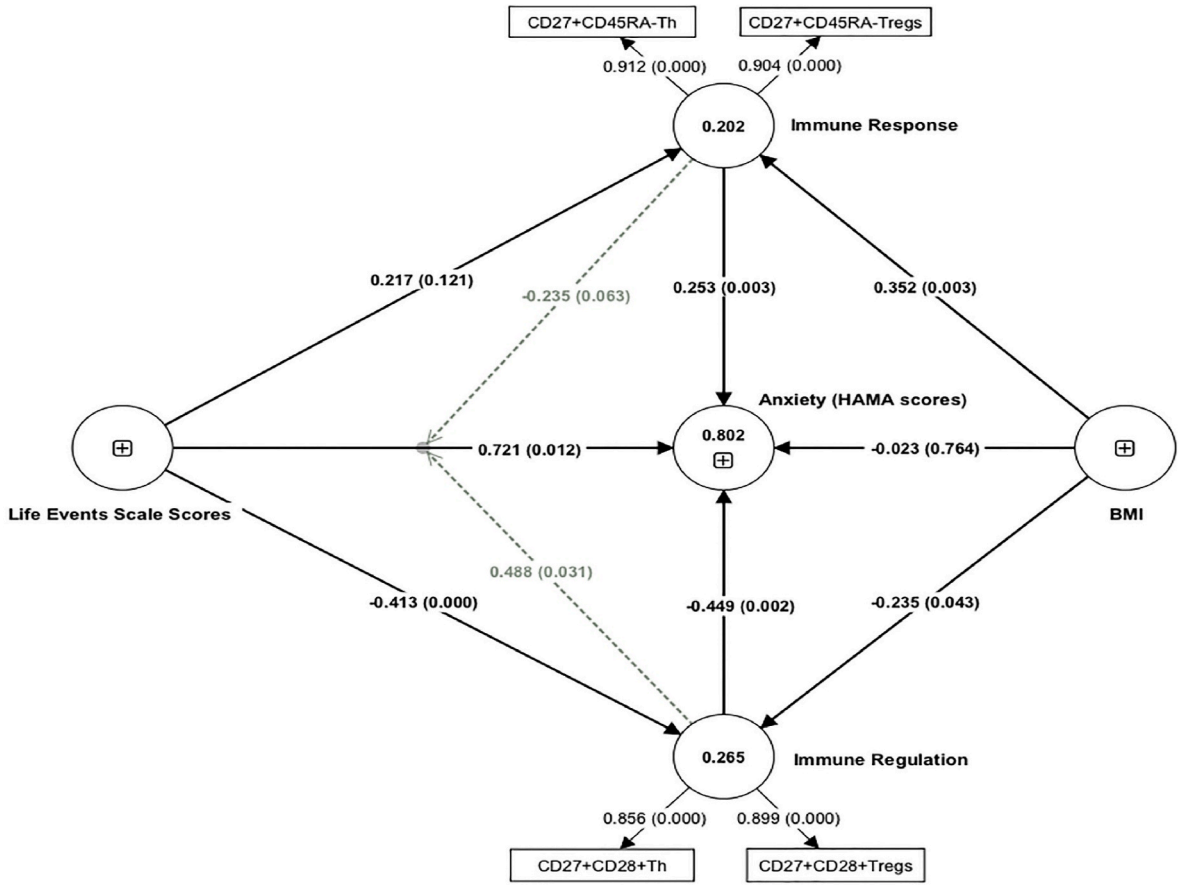

Fig. 5. Results of Partial Least Squares (PLS)-SEM analysis with the Anxiety (total HAMA score) as output variable, the adjusted mediating effects model. The immune response as indicated by a latent vector extracted from $CD27^+CD45RA^-$ Th and $CD27^+CD45RA^-$ Treg cells, the immune regulation as indicated by the latent vector extracted from $CD27^+CD28^+$ Tregs and $CD27^+CD28^+$ Th cells. The path coefficients or latent vector loadings were shown and accompanied by p-values. The interaction of the immune system with chronic psychological stress on anxiety was shown with the dashed line from the node of immune-related latent to the edge from LES to anxiety. BMI: Body Mass Index; HAMA: Hamilton anxiety scale.

molecular features of Th17 cells (Paroni et al., 2017). Indicating central memory $CD4^+$ T cells may have the capacity to induce Th17-like inflammatory response, which is involved in the generation of anxiety (Osborne et al., 2019).

The role of regulatory $CD4^+$ T cells in anxiety disorders has been investigated but is still not fully understood. A study of the anxiolytic effects of *Lactobacillus rhamnosus*(JB-1) found that depletion of regulatory T cells (Tregs, $CD4^+CD25^+$ T cells) suppressed the anxiolytic effects of JB-1, showing that $CD4^+CD25^+$ T cells may regulate anxiety through the number of Ly6Chi monocytes(Liu et al., 2020). Chronic social defeat stress increases peripheral Ly6Chi monocyte recruitment to the brain via IL-6-induced amplification of the neuroinflammatory response to stress, promoting anxiety-like behaviors(Niraula et al., 2019). Previous studies have shown that Tregs also secrete pro-inflammatory cytokines, including IL-13, IFN-γ, IL-1β, IL-17and other cytokines (Dominguez-Villar and Hafler, 2018; Kitz and Dominguez-Villar, 2017). IL-1β can activate microglia to activate TLR2/4 further, leading to abnormal activation of the medial prefrontal cortex (mPFC)-nucleus accumbens (NAc) neural circuit (Nie et al., 2018; Zou et al., 2022). Activation of the NAc may induce anxiety by over-activating dopaminergic neurons projecting to the ventral tegmental area (VTA) (Qi et al., 2022). Depletion of $Foxp3^+CD4^+$ Tregs is proven to be linked to the generation of anxiety-like behavior by abnormal regulation of peripheral immune response (Yang et al., 2023), demonstrating that peripheral immune homeostasis regulating by $CD4^+$ Treg cells may be a vital component of the body-mind interaction mechanism in anxiety disorders.

In this study, we found the proportion of $CD27^+CD28^+$ Th/Tregs inversely mediate the effect of stressful life events on anxiety. The primary role of CD28 as a costimulatory molecule in T cells is to promote T-cell activation and maintain proliferation(Hendriks et al., 2003), while loss of CD28 has been recognized as a reliable aging marker on T cells (Broux et al., 2012). In Tregs, CD28 plays an essential role in maintaining proliferation, and the lack of CD28 Tregs can lead to an increase in the level of activated $CD44^+$ T cells, which can lead to autoimmune reactions (Zhang et al., 2013). Increased peripheral $CD4^+CD28^{null}$ T cells as a pro-inflammatory phenotype of $CD4^+$ T cells can migrate towards the central nervous system and aggravate autoimmune inflammation, indicating that $CD4^+CD28^{null}$ T cells act on the process of neuroinflammation (Vanheusden et al., 2017). We postulated that $CD27^+CD28^+$ Th/Tregs may be involved in anxiety generation by regulating the complicated network of hemostasis of $CD4^+$ T cells. However, so far, the precise mechanism of different CD4CD28 T-cell subsets in the development of anxiety is still not fully understood.

4.1. Limitations and future research directions

The results of this study are consistent with previous studies that demonstrated changes in $CD4^+$ T-cell subsets may be associated with the pathophysiology of anxiety disorders. However, several key issues that remain unclear need to be investigated in the future. First, the molecular and neural circuits mechanism of the decreased $CD4^+CD27^+CD28^+$ T-cell subsets in the onset of anxiety need to be elucidated to help us understand the details of the immune-related pathophysiology of anxiety disorders. Second, it is necessary to test and validate whether

$CD4^+CD27^+CD28^+$ T-cell subsets can be applied as robust biomarkers for the diagnosis of anxiety disorders or prediction for treatment efficacy by a large prospective multicenter study. Moreover, whether $CD4^+CD27^+CD28^+$ T-cell subsets can be the potentially interventional target for anxiety disorders deserves further exploration.

Finally, we noted that this study has several limitations which should be considered when interpreting the results. With the relaxation of the "zero-COVID" prevention and control policy in mainland China, the surge in COVID-19 infections at the end of 2022 impacted the recruitment of subjects, resulting in a small sample size after applying the inclusion and exclusion criteria. Previous studies indicated that the frequency of peripheral naïve, memory, as well as effector $CD4^+$ T cell subsets, are associated with the change in BMI (Elegido et al., 2017; Ilavská et al., 2012; Pangrazzi et al., 2020). However, we did not control for covariates of BMI when comparing the subsets of CD4+T cells between case and control, indicating that it needs to be noted in the study design to prevent the influence of potential confounding variates in future studies. Additionally, this study had a single-center design, which may impact the representativeness of the data. Due to the limitations of a cross-sectional design, we cannot directly draw causal conclusions. A larger sample cohort and prospective study are needed to verify the results of this study further.

4.2. Conclusion

In summary, our study comprehensively evaluated the detail of differential $CD4^+$ T-cell subsets, including Th and Treg cells, in newly diagnosed and drug naïve anxiety disorders patients, confirming the imbalance of effector and regulatory $CD4^+$ T-cell population in anxiety disorders. We also test the interaction between the $CD4^+$ T cells and long-term psychological stress on anxiety, indicating that the immune regulation population involved in $CD27^+CD28^+$Th/Treg cells plays an essential role in mediating the effect of chronic stress on anxiety. Furthermore, this study provides a clinical basis for further exploration of the role and molecular mechanism of CD4CD27CD28 T cell subsets in anxiety disorders.

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CRediT authorship contribution statement

Bindong Dai: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Tao Li:** Resources. **Jinya Cao:** Resources. **Xiaohui Zhao:** Resources. **Yinan Jiang:** Resources. **Lili Shi:** Resources. **Jing Wei:** Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2024.100661.

References

Ahmed, R., Bevan, M.J., Reiner, S.L., Fearon, D.T., 2009. The precursors of memory: models and controversies. Nat. Rev. Immunol. 9 (9), 662–668. https://doi.org/10.1038/nri2619.

Biltz, R.G., Sawicki, C.M., Sheridan, J.F., Godbout, J.P., 2022. The neuroimmunology of social-stress-induced sensitization. Nat. Immunol. 23 (11), 1527–1535. https://doi.org/10.1038/s41590-022-01321-z.

Broux, B., Markovic-Plese, S., Stinissen, P., Hellings, N., 2012. Pathogenic features of CD4+CD28- T cells in immune disorders. Trends Mol. Med. 18 (8), 446–453. https://doi.org/10.1016/j.molmed.2012.06.003.

Chan, K.L., Poller, W.C., Swirski, F.K., Russo, S.J., 2023. Central regulation of stress-evoked peripheral immune responses. Nat. Rev. Neurosci. 24 (10), 591–604. https://doi.org/10.1038/s41583-023-00729-2.

Cohen, R.I., Ye, X., Ramdeo, R., Liu, S.F., 2019. The number and function of T regulatory cells in obese atopic female asthmatics. J. Asthma 56 (3), 303–310. https://doi.org/10.1080/02770903.2018.1452935.

Craske, M.G., Stein, M.B., Eley, T.C., Milad, M.R., Holmes, A., Rapee, R.M., Wittchen, H. U., 2017. Anxiety disorders. Nat. Rev. Dis. Prim. 3, 17024 https://doi.org/10.1038/nrdp.2017.24.

de Graaf, M.T., Smitt, P.A., Luitwieler, R.L., van Velzen, C., van den Broek, P.D., Kraan, J., Gratama, J.W., 2011. Central memory CD4+ T cells dominate the normal cerebrospinal fluid. Cytometry B Clin Cytom 80 (1), 43–50. https://doi.org/10.1002/cyto.b.20542.

de Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6 (6), 463–475. https://doi.org/10.1038/nrn1683.

Deets, K.A., Vance, R.E., 2021. Inflammasomes and adaptive immune responses. Nat. Immunol. 22 (4), 412–422. https://doi.org/10.1038/s41590-021-00869-6.

Dominguez-Villar, M., Hafler, D.A., 2018. Regulatory T cells in autoimmune disease. Nat. Immunol. 19 (7), 665–673. https://doi.org/10.1038/s41590-018-0120-4.

Elegido, A., Graell, M., Andrés, P., Gheorghe, A., Marcos, A., Nova, E., 2017. Increased naive CD4(+) and B lymphocyte subsets are associated with body mass loss and drive relative lymphocytosis in anorexia nervosa patients. Nutr. Res. 39, 43–50. https://doi.org/10.1016/j.nutres.2017.02.006.

Engelhardt, B., Ransohoff, R.M., 2005. The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. Trends Immunol. 26 (9), 485–495. https://doi.org/10.1016/j.it.2005.07.004.

Engler, H., Brendt, P., Wischermann, J., Wegner, A., Röhling, R., Schoemberg, T., Schedlowski, M., 2017. Selective increase of cerebrospinal fluid IL-6 during experimental systemic inflammation in humans: association with depressive symptoms. Mol. Psychiatr. 22 (10), 1448–1454. https://doi.org/10.1038/mp.2016.264.

Evans, F.L., Dittmer, M., de la Fuente, A.G., Fitzgerald, D.C., 2019. Protective and regenerative roles of T cells in central nervous system disorders. Front. Immunol. 10, 2171. https://doi.org/10.3389/fimmu.2019.02171.

Fan, K.Q., Li, Y.Y., Wang, H.L., Mao, X.T., Guo, J.X., Wang, F., Jin, J., 2019. Stress-induced metabolic disorder in peripheral CD4(+) T cells leads to anxiety-like behavior. Cell 179 (4), 864–879.e819. https://doi.org/10.1016/j.cell.2019.10.001.

Fernandes, D.J., Spring, S., Corre, C., Tu, A., Qiu, L.R., Hammill, C., Lerch, J.P., 2022. Mouse models of immune dysfunction: their neuroanatomical differences reflect their anxiety-behavioural phenotype. Mol. Psychiatr. 27 (7), 3047–3055. https://doi.org/10.1038/s41380-022-01535-5.

Filiano, A.J., Xu, Y., Tustison, N.J., Marsh, R.L., Baker, W., Smirnov, I., Kipnis, J., 2016. Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour. Nature 535 (7612), 425–429. https://doi.org/10.1038/nature18626.

Fritsch, R.D., Shen, X., Illei, G.G., Yarboro, C.H., Prussin, C., Hathcock, K.S., Lipsky, P.E., 2006. Abnormal differentiation of memory T cells in systemic lupus erythematosus. Arthritis Rheum. 54 (7), 2184–2197. https://doi.org/10.1002/art.21943.

Global, regional, and national burden of 12 mental disorders in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Psychiatr. 9 (2), 2022, 137–150. https://doi.org/10.1016/s2215-0366(21)00395-3.

Gouin, J.P., Hantsoo, L., Kiecolt-Glaser, J.K., 2008. Immune dysregulation and chronic stress among older adults: a review. Neuroimmunomodulation 15 (4–6), 251–259. https://doi.org/10.1159/000156468.

Gu, X.X., Jin, Y., Fu, T., Zhang, X.M., Li, T., Yang, Y., Gu, Z.F., 2021. Relevant characteristics analysis using natural language processing and machine learning based on phenotypes and T-cell subsets in systemic lupus erythematosus patients with anxiety. Front. Psychiatr. 12, 793505 https://doi.org/10.3389/fpsyt.2021.793505.

Hamilton, M., 1959. The assessment of anxiety states by rating. Br. J. Med. Psychol. 32 (1), 50–55. https://doi.org/10.1111/j.2044-8341.1959.tb00467.x.

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry 23 (1), 56–62. https://doi.org/10.1136/jnnp.23.1.56.

Haroon, E., Raison, C.L., Miller, A.H., 2012. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 37 (1), 137–162. https://doi.org/10.1038/npp.2011.205.

Haykin, H., Rolls, A., 2021. The neuroimmune response during stress: a physiological perspective. Immunity 54 (9), 1933–1947. https://doi.org/10.1016/j.immuni.2021.08.023.
Hendriks, J., Xiao, Y., Borst, J., 2003. CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. J. Exp. Med. 198 (9), 1369–1380. https://doi.org/10.1084/jem.20030916.
Hintzen, R.Q., Lens, S.M., Lammers, K., Kuiper, H., Beckmann, M.P., van Lier, R.A., 1995. Engagement of CD27 with its ligand CD70 provides a second signal for T cell activation. J. Immunol. 154 (6), 2612–2623.
Ilavská, S., Horváthová, M., Szabová, M., Nemessányi, T., Jahnová, E., Tulinská, J., Volkovová, K., 2012. Association between the human immune response and body mass index. Hum. Immunol. 73 (5), 480–485. https://doi.org/10.1016/j.humimm.2012.02.023.
Kalin, N.H., 2020. The critical relationship between anxiety and depression. Am. J. Psychiatr. 177 (5), 365–367. https://doi.org/10.1176/appi.ajp.2020.20030305.
Kitz, A., Dominguez-Villar, M., 2017. Molecular mechanisms underlying Th1-like Treg generation and function. Cell. Mol. Life Sci. 74 (22), 4059–4075. https://doi.org/10.1007/s00018-017-2569-y.
Klopack, E.T., Crimmins, E.M., Cole, S.W., Seeman, T.E., Carroll, J.E., 2022. Social stressors associated with age-related T lymphocyte percentages in older US adults: evidence from the US Health and Retirement Study. Proc. Natl. Acad. Sci. U.S.A. 119 (25), e2202780119 https://doi.org/10.1073/pnas.2202780119.
Kovaiou, R.D., Weiskirchner, I., Keller, M., Pfister, G., Cioca, D.P., Grubeck-Loebenstein, B., 2005. Age-related differences in phenotype and function of CD4+ T cells are due to a phenotypic shift from naive to memory effector CD4+ T cells. Int. Immunol. 17 (10), 1359–1366. https://doi.org/10.1093/intimm/dxh314.
Kumar, B.V., Connors, T.J., Farber, D.L., 2018. Human T cell development, localization, and function throughout life. Immunity 48 (2), 202–213. https://doi.org/10.1016/j.immuni.2018.01.007.
Kunkl, M., Frascolla, S., Amormino, C., Volpe, E., Tuosto, L., 2020. T helper cells: the modulators of inflammation in multiple sclerosis. Cells 9 (2). https://doi.org/10.3390/cells9020482.
Lenschow, D.J., Walunas, T.L., Bluestone, J.A., 1996. CD28/B7 system of T cell costimulation. Annu. Rev. Immunol. 14, 233–258. https://doi.org/10.1146/annurev.immunol.14.1.233.
Leonard, B.E., Song, C., 1996. Stress and the immune system in the etiology of anxiety and depression. Pharmacol. Biochem. Behav. 54 (1), 299–303. https://doi.org/10.1016/0091-3057(95)02158-2.
Liu, Y., Mian, M.F., McVey Neufeld, K.A., Forsythe, P., 2020. CD4(+)CD25(+) T cells are essential for behavioral effects of Lactobacillus rhamnosus JB-1 in male BALB/c mice. Brain Behav. Immun. 88, 451–460. https://doi.org/10.1016/j.bbi.2020.04.014.
Lin, J.Y., Huang, Y., Su, Y.A., Yu, X., Lyu, X.Z., Liu, Q., Si, T.M., 2018. Association between perceived stressfulness of stressful life events and the suicidal risk in Chinese patients with major depressive disorder. Chin. Med. J. 131 (8), 912–919. https://doi.org/10.4103/0366-6999.229898.
Ma, Z., Zhao, M., Zhao, H., Qu, N., 2023. Causal role of immune cells in generalized anxiety disorder: mendelian randomization study. Front. Immunol. 14, 1338083 https://doi.org/10.3389/fimmu.2023.1338083.
Malek, M.H., Berger, D.E., Coburn, J.W., 2007. On the inappropriateness of stepwise regression analysis for model building and testing. Eur. J. Appl. Physiol. 101 (2), 263–264. https://doi.org/10.1007/s00421-007-0485-9 author reply 265-266.
McKim, D.B., Weber, M.D., Niraula, A., Sawicki, C.M., Liu, X., Jarrett, B.L., Godbout, J.P., 2018. Microglial recruitment of IL-1β-producing monocytes to brain endothelium causes stress-induced anxiety. Mol. Psychiatr. 23 (6), 1421–1431. https://doi.org/10.1038/mp.2017.64.
Michopoulos, V., Powers, A., Gillespie, C.F., Ressler, K.J., Jovanovic, T., 2017. Inflammation in fear- and anxiety-based disorders: PTSD, GAD, and beyond. Neuropsychopharmacology 42 (1), 254–270. https://doi.org/10.1038/npp.2016.146.
Nautiyal, K.M., Ribeiro, A.C., Pfaff, D.W., Silver, R., 2008. Brain mast cells link the immune system to anxiety-like behavior. Proc. Natl. Acad. Sci. U.S.A. 105 (46), 18053–18057. https://doi.org/10.1073/pnas.0809479105.
Nie, X., Kitaoka, S., Tanaka, K., Segi-Nishida, E., Imoto, Y., Ogawa, A., Furuyashiki, T., 2018. The innate immune receptors TLR2/4 mediate repeated social defeat stress-induced social avoidance through prefrontal microglial activation. Neuron 99 (3), 464–479.e467. https://doi.org/10.1016/j.neuron.2018.06.035.
Niraula, A., Witcher, K.G., Sheridan, J.F., Godbout, J.P., 2019. Interleukin-6 induced by social stress promotes a unique transcriptional signature in the monocytes that facilitate anxiety. Biol. Psychiatr. 85 (8), 679–689. https://doi.org/10.1016/j.biopsych.2018.09.030.
Nishihara, H., Soldati, S., Mossu, A., Rosito, M., Rudolph, H., Muller, W.A., Engelhardt, B., 2020. Human CD4(+) T cell subsets differ in their abilities to cross endothelial and epithelial brain barriers in vitro. Fluids Barriers CNS 17 (1), 3. https://doi.org/10.1186/s12987-019-0165-2.
Okada, R., Kondo, T., Matsuki, F., Takata, H., Takiguchi, M., 2008. Phenotypic classification of human CD4+ T cell subsets and their differentiation. Int. Immunol. 20 (9), 1189–1199. https://doi.org/10.1093/intimm/dxn075.
Ortega, S.B., Torres, V.O., Latchney, S.E., Whoolery, C.W., Noorbhai, I.Z., Poinsatte, K., Stowe, A.M., 2020. B cells migrate into remote brain areas and support neurogenesis and functional recovery after focal stroke in mice. Proc. Natl. Acad. Sci. U.S.A. 117 (9), 4983–4993. https://doi.org/10.1073/pnas.1913292117.
Osborne, L.M., Brar, A., Klein, S.L., 2019. The role of Th17 cells in the pathophysiology of pregnancy and perinatal mood and anxiety disorders. Brain Behav. Immun. 76, 7–16. https://doi.org/10.1016/j.bbi.2018.11.015.
Pangrazzi, L., Naismith, E., Miggitsch, C., Carmona Arana, J.A., Keller, M., Grubeck-Loebenstein, B., Weinberger, B., 2020. The impact of body mass index on adaptive immune cells in the human bone marrow. Immun. Ageing 17, 15. https://doi.org/10.1186/s12979-020-00186-w.
Paroni, M., Maltese, V., De Simone, M., Ranzani, V., Larghi, P., Fenoglio, C., Geginat, J., 2017. Recognition of viral and self-antigens by T(H)1 and T(H)1/T(H)17 central memory cells in patients with multiple sclerosis reveals distinct roles in immune surveillance and relapses. J. Allergy Clin. Immunol. 140 (3), 797–808. https://doi.org/10.1016/j.jaci.2016.11.045.
Pasciuto, E., Burton, O.T., Roca, C.P., Lagou, V., Rajan, W.D., Theys, T., Liston, A., 2020. Microglia require CD4 T cells to complete the fetal-to-adult transition. Cell 182 (3), 625–640.e624. https://doi.org/10.1016/j.cell.2020.06.026.
Penninx, B.W., Pine, D.S., Holmes, E.A., Reif, A., 2021. Anxiety disorders. Lancet 397 (10277), 914–927. https://doi.org/10.1016/s0140-6736(21)00359-7.
Pepper, M., Jenkins, M.K., 2011. Origins of CD4(+) effector and central memory T cells. Nat. Immunol. 12 (6), 467–471. https://doi.org/10.1038/ni.2038.
Pérez-Pérez, B., Cristóbal-Narváez, P., Sheinbaum, T., Kwapil, T.R., Ballespí, S., Peña, E., Barrantes-Vidal, N., 2018. Interaction between FKBP5 variability and recent life events in the anxiety spectrum: evidence for the differential susceptibility model. PLoS One 13 (2), e0193044. https://doi.org/10.1371/journal.pone.0193044.
Prather, A.A., Epel, E.S., Portela Parra, E., Coccia, M., Puterman, E., Aiello, A.E., Dhabhar, F.S., 2018. Associations between chronic caregiving stress and T cell markers implicated in immunosenescence. Brain Behav. Immun. 73, 546–549. https://doi.org/10.1016/j.bbi.2018.06.019.
Qi, G., Zhang, P., Li, T., Li, M., Zhang, Q., He, F., Tian, B., 2022. NAc-VTA circuit underlies emotional stress-induced anxiety-like behavior in the three-chamber vicarious social defeat stress mouse model. Nat. Commun. 13 (1), 577. https://doi.org/10.1038/s41467-022-28190-2.
Quagliato, L.A., Nardi, A.E., 2018. Cytokine alterations in panic disorder: a systematic review. J. Affect. Disord. 228, 91–96. https://doi.org/10.1016/j.jad.2017.11.094.
Rattazzi, L., Piras, G., Ono, M., Deacon, R., Pariante, C.M., D'Acquisto, F., 2013. CD4$^+$ but not CD8$^+$ T cells revert the impaired emotional behavior of immunocompromised RAG-1-deficient mice. Transl. Psychiatry 3 (7), e280. https://doi.org/10.1038/tp.2013.54.
Riou, J., Guyon, H., Falissard, B., 2016. An introduction to the partial least squares approach to structural equation modelling: a method for exploratory psychiatric research. Int. J. Methods Psychiatr. Res. 25 (3), 220–231. https://doi.org/10.1002/mpr.1497.
Rodriguez, IJ, Lalinde Ruiz, N, Llano León, M, Martínez Enríquez, L, Montilla Velásquez, MDP, Ortiz Aguirre, JP, et al., 2020. Immunosenescence Study of T Cells: A Systematic Review. Front. Immunol. 11, 604591.
Sahir, F., Mateo, J.M., Steinhoff, M., Siveen, K.S., 2020. Development of a 43 color panel for the characterization of conventional and unconventional T-cell subsets, B cells, NK cells, monocytes, dendritic cells, and innate lymphoid cells using spectral flow cytometry. Cytometry. https://doi.org/10.1002/cyto.a.24288.
Sallusto, F., Geginat, J., Lanzavecchia, A., 2004. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu. Rev. Immunol. 22, 745–763. https://doi.org/10.1146/annurev.immunol.22.012703.104702.
Serre-Miranda, C., Roque, S., Santos, N.C., Portugal-Nunes, C., Costa, P., Palha, J.A., Correia-Neves, M., 2015. Effector memory CD4(+) T cells are associated with cognitive performance in a senior population. Neurol Neuroimmunol Neuroinflamm 2 (1), e54. https://doi.org/10.1212/nxi.0000000000000054.
Shen, Z., Cui, L., Mou, S., Ren, L., Yuan, Y., Shen, X., Li, G., 2022. Combining S100B and cytokines as neuro-inflammatory biomarkers for diagnosing generalized anxiety disorder: a proof-of-concept study based on machine learning. Front. Psychiatr. 13, 881241 https://doi.org/10.3389/fpsyt.2022.881241.
Straub, R.H., Cutolo, M., 2018. Psychoneuroimmunology-developments in stress research. Wien Med. Wochenschr. 168 (3–4), 76–84. https://doi.org/10.1007/s10354-017-0574-2.
Szuhany, K.L., Simon, N.M., 2022. Anxiety disorders: a review. JAMA 328 (24), 2431–2445. https://doi.org/10.1001/jama.2022.22744.
Tang, Z., Ye, G., Chen, X., Pan, M., Fu, J., Fu, T., Hou, R., 2018. Peripheral proinflammatory cytokines in Chinese patients with generalised anxiety disorder. J. Affect. Disord. 225, 593–598. https://doi.org/10.1016/j.jad.2017.08.082.
Thompson, E., 2015. Hamilton rating scale for anxiety (HAM-A). Occup. Med. (Lond.) 65 (7), 601. https://doi.org/10.1093/occmed/kqv054.
Vanheusden, M., Broux, B., Welten, S.P.M., Peeters, L.M., Panagioti, E., Van Wijmeersch, B., Hellings, N., 2017. Cytomegalovirus infection exacerbates autoimmune mediated neuroinflammation. Sci. Rep. 7 (1), 663. https://doi.org/10.1038/s41598-017-00645-3.
Vieira, M.M., Ferreira, T.B., Pacheco, P.A., Barros, P.O., Almeida, C.R., Araújo-Lima, C.F., Bento, C.A., 2010. Enhanced Th17 phenotype in individuals with generalized anxiety disorder. J. Neuroimmunol. 229 (1–2), 212–218. https://doi.org/10.1016/j.jneuroim.2010.07.018.
Vogelzangs, N., Beekman, A.T., de Jonge, P., Penninx, B.W., 2013. Anxiety disorders and inflammation in a large adult cohort. Transl. Psychiatry 3 (4), e249. https://doi.org/10.1038/tp.2013.27.
Wiley, K.S., Kwon, D., Knorr, D.A., Fox, M.M., 2024. Regulatory T-cell phenotypes in prenatal psychological distress. Brain Behav. Immun. 116, 62–69. https://doi.org/10.1016/j.bbi.2023.11.033.
Yang, E.J., Rahim, M.A., Griggs, E., Iban-Arias, R., Pasinetti, G.M., 2023. Transient anxiety-and depression-like behaviors are linked to the depletion of Foxp3-expressing cells via inflammasome in the brain. PNAS Nexus 2 (8), pgad251. https://doi.org/10.1093/pnasnexus/pgad251.

Zhang, R., Huynh, A., Whitcher, G., Chang, J., Maltzman, J.S., Turka, L.A., 2013. An obligate cell-intrinsic function for CD28 in Tregs. J. Clin. Invest. 123 (2), 580–593. https://doi.org/10.1172/jci65013.

Zheng, Y.P., Lin, K.M., 1994. A nationwide study of stressful life events in Mainland China. Psychosom. Med. 56 (4), 296–305. https://doi.org/10.1097/00006842-199407000-00004.

Zheng, Z.H., Tu, J.L., Li, X.H., Hua, Q., Liu, W.Z., Liu, Y., Zhang, W.H., 2021. Neuroinflammation induces anxiety- and depressive-like behavior by modulating neuronal plasticity in the basolateral amygdala. Brain Behav. Immun. 91, 505–518. https://doi.org/10.1016/j.bbi.2020.11.007.

Zhu, L., Song, H., Zhang, L., Meng, H., 2019. Characterization of IL-17-producing Treg cells in type 2 diabetes patients. Immunol. Res. 67 (4–5), 443–449. https://doi.org/10.1007/s12026-019-09095-7.

Zou, J., Shang, W., Yang, L., Liu, T., Wang, L., Li, X., Fan, X., 2022. Microglia activation in the mPFC mediates anxiety-like behaviors caused by Staphylococcus aureus strain USA300. Brain Behav 12 (9), e2715. https://doi.org/10.1002/brb3.2715.