BMC Psychiatry

RESEARCH

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Correlations between major depressive disorder, splenic morphology, and immune function

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Abstract

To analyze the symptoms, courses, and severities of depressive disorder, as well as the morphological changes in the spleens and related immune mechanisms, we recruited patients with first-episode or recurrent major depressive disorder (MDD) (patient group) and healthy controls (normal group) matched in age and gender. We measured their plasma MICB (pg/ml), ULBP1 (ng/ml), and splenic volume (cm^3) at baseline. The patient group was randomly assigned to receive (S)-ketamine (study group) or saline (control group), and the above indices were collected again on the 4th weekend after administration. At baseline, both MICB and splenic volume were significantly higher in the patient group than in the normal group. A positive correlation was observed between MICB and splenic volume in the patient group. After (S)-ketamine administration, the elevated splenic volume and MICB levels decreased. These results suggest that the pathogenesis of MDD may involve abnormal MICB expression and splenic morphology. (S)-ketamine may ameliorate inflammation and enhance splenic function, thereby relieving MDD symptoms.

Highlights

- Abnormal immune functions are associated with MDD, characterized mainly by abnormal MICB expression and splenic morphology.
- The spleen may be involved in the pathogenesis of depression through regulating MICB-NKG2D.
- (S)-ketamine may relieve inflammation and improve splenic function, thereby promoting short-term recovery in patients.

Keywords Major depressive disorder, (S)-ketamine, MICB, Splenic morphology

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Background

Stress can disrupt immunity to weaken the host's defense against diseases [1]. Accumulating evidence suggests that the immune system may be involved in the pathogenesis of stress-related mental disorders, such as depression [2–5]. Immunity affects how the brain regulates emotional and behavioral processes. Cytokines in the immune system participate in neuroinflammation and neurodegeneration [1]. A recent study suggests that the central nervous system of hamsters, infected with severe acute respiratory syndrome coronavirus 2, exhibits abnormal microglial activation, macrophage infiltration, as well as excessive type I interferon and chemokine responses, all of which interact to cause immune system dysfunction [6]. This immune system dysfunction further evokes arousal, cognitive deficits, executive disorders, and even emotional disorders such as major depressive disorder (MDD)[6]. These disorders also develop as the hypothalamic-pituitary-adrenocortical axis, or corticotropin and monoamine metabolism, is distorted in an abnormal immune system [3, 7, 8]. Additionally, some immune-related cytokines play a prominent role in the pathogenesis of mental disorders. For example, IL- 6, IL- 10 and TNF-α are upregulated during the course of MDD [9, 10].

The spleen, responsible for neuroimmune regulation, communicates with the brain through the autonomic nervous system [11]. As the largest lymphoid organ of the human body, the spleen generates immune cells that capture pathogens and antigens to trigger innate and adaptive immune responses. Animal studies have shown that the emotional behaviors of mice under acute stress are correlated with the transport of monocytes from the spleen to the brain [12]. Additionally, the expression levels of IL- 6 and TNF-α in the spleen of animal models of depression are significantly upregulated [13]. These studies suggest a dynamic interaction between the autonomic nervous system and the spleen. Some scholars attribute this interaction to the regulatory role of the brain-spleen axis [14]. An animal study has shown that the protein level of natural killer group 2, member D (NKG2D) and the number of granulocytes in the spleen, the splenic volume and weight are higher in chronic social defeat stress (CSDS)-susceptible mice than in normal mice and CSDS-resilient mice [15]. Additionally, NKG2D expression elevates in the postmortem spleen tissues of MDD patients [15]. These research outcomes may indicate a correlation among NKG2D, spleen and mental disorders such as depression.

NKG2D and its ligands are integral components of the immune system, and act as an effective defense against tumors and immune system diseases [16, 17]. NKG2D can be activated on NK cells, cytotoxic T cells, and other T cell subsets to eliminate ligand-carrying cells and produce pro-inflammatory cytokines [18–22]. NKG2D ligands include major histocompatibility complex class I chain-related proteins A and B (MICA/MICB) and UL binding proteins (ULBP)1–6 [16, 23]. ULBP1 -6 shares functions with MICA and MICB, but has a different structure [23]. NKG2D ligands are lowly expressed in some normal cells, but positively expressed under stimuli such as tumors or viruses [22]. However, previous studies have not revealed the relationship between MDD and MICB or ULBP1.

Since 2000, multiple studies have confirmed the powerful antidepressant and anti-suicide effects of ketamine in patients with MDD or bipolar disorder [24–27]. A slow infusion of ketamine (0.5 mg/kg over 40 min) significantly reduces depressive symptoms lasting about one week [28]. Ketamine is a racemic mixture consisting of two enantiomers, (R)-ketamine and (S)-ketamine. Similar to ketamine, (S)-ketamine is definitely effective, safe and well-tolerated in relieving depression [29]. The antidepressant mechanism of ketamine and its enantiomers remains unclear. Neurophysiological evidence suggests that changes in frontoparietal connectivity and alterations in GABA-A receptor function underpin the antidepressant effects of ketamine [30]. These effects may be related to the improvement on the metabolism of the right habenula and on the function of the medial and orbitofrontal cortex networks [31]. Additionally, ketamine may produce antidepressant effects by activating the D1 receptor to partially restore synaptic plasticity in the nucleus accumbens [32]. As demonstrated by Zhang et al., ketamine can reverse CSCD-induced increases in NKG2D protein levels, granulocyte count and spleen weight in mice [15]. Another study shows that chronic variable stress (CVS) induces depression and anxiety-like behavior, as well as significant changes in plasma inflammatory factors of mice; but after the administration of (S)-ketamine, antidepressant and anti-anxiety effects are observed, and the inflammatory disturbances caused by CVS are also partially restored [33]. The complement system is a vital in the innate immune response and crucial for synaptic plasticity. Some scholars propose that ketamine may exert its antidepressant effects by regulating the complement system [34]. These studies indicate that the antidepressant mechanism of ketamine may also involve immune responses.

Therefore, we are encouraged to investigate whether certain immunological features, such as plasma levels of MICB and ULBP1 and spleen volume, are altered in patients with MDD, and whether S-ketamine has an effect on these features. In this study, we hypothesized that the immune system takes on a different profile in patients with MDD, and the plasma levels of some cytokines, such as MICB and ULBP1, may be dysregulated to change the

morphology of the spleen. The purpose of this 4-week study was to investigate whether there were abnormalities in plasma MICB and ULBP1 levels and spleen morphology in patients with severe depression, and whether S-ketamine combined with standardized antidepressants could improve the above indexes.

Methods

Patients and enrollment

From October 2020 to December 2022, we recruited patients with first-episode or recurrent MDD and sex- and age-matched controls at Wuxi Mental Health Center and Wuxi Hospital of Traditional Chinese Medicine. All the participants had normal audio-visual functions, as well as abilities to comprehend and complete tests.

The criteria for enrolling patients were as follows: (1) meeting the diagnostic criteria for MDD in the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM- 5), and not meeting any other psychiatric diagnosis; (2) taking no antidepressants if the patient was in the first episode, or in the previous 4 weeks if the patient was in relapsed MDD. The criteria for enrolling normal controls were as follows: not meeting any diagnostic criteria for depressive disorder or other mental disorders in DSM- 5. The common inclusion criteria for patients and normal controls were as follows: age between 18 and 65 years old, either gender, Han Chinese. The common exclusion criteria for patients and normal controls were: (1) severe medical and surgical diseases, nervous system diseases; (2) abnormal laboratory tests (e.g., blood routine, liver and kidney function, etc.); (3) pregnancy or lactation; (4) participation in clinical trials of other drugs in the previous three months; (5) recent severe infection, and receipt of drugs that might affect immune function; (6) substance dependence or abusing (including tobacco and alcohol). Additionally, to minimize the potential confounding effects of height and weight on spleen volume, we endeavored to select subjects with comparable height and weight profiles [35, 36].

This study was approved by the Ethics Committee of Wuxi Mental Health Center (Batch Number: WXMHCIRB2020LLky039). All subjects provided informed consent.

Study design

This was the first controlled study on the relationship between MICB, ULBP1, and spleen morphology in MDD patients. Given the limited existing data on intervention effects, the sample size was determined based on the minimum required for significant differences in scale scores after treatment with (S)-ketamine and the minimum sample size required for t-tests. We first compared the plasma MICB and ULBP1 levels and the splenic volume of MDD patients (patient group) and healthy controls (normal group). Then, the patient group was randomly divided into the study group and the control group. The study group was given (S)-ketamine, and the control group was given saline. The patient group was given selective serotonin reuptake inhibitors (SSRIs) and/or selective serotonin and norepinephrine reuptake inhibitors (SNRIs) for standardized antidepressant treatment. Specifically, 16 cases received escitalopram (10–20 mg/day), 25 cases received sertraline (50–200 mg/day), 14 cases received fluvoxamine (100–300 mg/day), 10 cases received duloxetine (40–60 mg/day), and 15 cases received combined medications. The healthy control group did not receive any drug intervention. The MICB, ULBP1, and splenic volume were compared between the study group and the control group at baseline and 4 weeks after administration. (Fig. 1).

Scales

General information was collected using a self-made scale, including name, sex, age, nationality, educational time, occupation, brief medical history, allergy history, surgical trauma history, medication history, tobacco and alcohol history, weight, height, and so on. Montgomery–Åsberg Depression Rating Scale (MADRS) was used to evaluate the symptoms and the effects of antidepressants [37]. The 6-item Clinician-Administered Dissociation Symptom Scale was utilized to evaluate dissociative symptoms [38]. A reduction of 30% or more in MADRS score was defined as effective, and cure as MADRS ≤ 10 [29, 39].

(S)-ketamine administration

One intravenous injection of (S)-ketamine was administered. To prevent accidental inhalation due to vomiting, patients were instructed to fast for 6 h before the start of administration. The dosage of (S)-ketamine (trade name: Esketamine Hydrochloride Injection, 2 ml:50 mg, Jiangsu Hengrui Pharmaceutical Co., Ltd.) was set at 0.4 mg/kg [29, 40]. An infusion of 0.9% sodium chloride (50 ml) was administered via a micropump over 50 min. The procedure was conducted by experienced nurses. During administration, patients inhaled oxygen (1–2 L/min), and blood pressure, pulse, respiratory rate, oxygen saturation, consciousness, and acute adverse reactions were monitored and recorded every 10 min.

Preparation of plasma samples and measurement of MICB and ULBP1

Blood samples were collected by venipuncture, separated twice and cryopreserved. For the patient group, blood was sampled once before and after treatment,

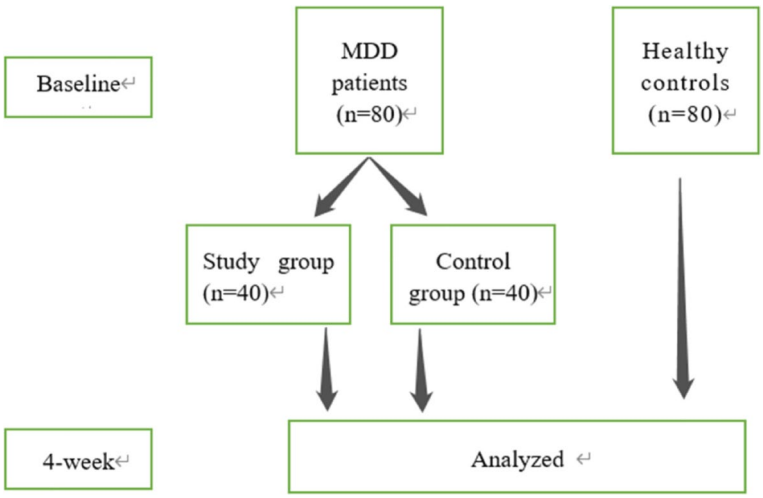

Fig. 1 Diagram for a controlled Study of administered (S)-ketamine for patients with MDD. Abbreviations: MDD, major depressive disorder. The study group received (S)-ketamine, while the control group received saline

respectively; for the normal group, blood was collected only once at baseline.

All plasma samples were analyzed using enzyme-linked immunosorbent assay (ELISA) to measure the concentrations of MICB and ULBP1. Human MICB ELISA Kit (96 T, American Thermo Scientific Company) and Human ULBP1 ELISA Kit (96 T, American Thermo Scientific Company) were used for this purpose [41, 42].

Measurement of splenic volume

Splenic volume was measured in the Radiology Department of Wuxi Mental Health Center using a PHILIPS 64-row 128-layer spiral CT, model Ingenuity Core 128 and workstation ADW4.5, with parameters set as follows: tube voltage (120 kV), tube current (143 mAs), rotation time (0.8 s), pitch (1.375), matrix (512 * 512), scanning range (500 mm), pixel (148%), display field of view (35.0 * 40.5 cm), layer spacing (3 mm). After spleen scanning, the images were reconstructed into slices 0.625 mm thick and sent to ADW4.5 workstation. All subjects were scanned for the spleen according to the same parameters, and one-dimensional measurement of spleen on coronal images were performed by qualified radiologists. The longest distance between the poles of the spleen was measured as its diameter and recorded manually. All CT image data were transferred to a workstation dedicated to 3D CT volume analysis. The volume and size of the spleen were measured by the same radiologist. Another radiologist analyzed the splenic volumes of randomly selected subjects to check for consistency [43].

Statistical analysis

IBM SPSS version 19.0 was used for statistical analysis. All measurement data were expressed as mean ± standard deviation (Mean ± SD). Two-Way Repeated Measures ANOVA was used to compare MADRS scores at different time points between the study group and the control group. To analyze changes in spleen volume and assess the effects of potential confounding factors, such as age, height and weight, on spleen volume, we used the following statistical methods. First, analysis of covariance (ANCOVA) was performed. Age, height, and weight were included as covariates in the model to control the potential effects of these factors on spleen volume. Spleen volume $=\beta_0 + \beta_1 \times$ treatment group effect $+\beta_2 \times$ age $+\beta_3 \times$ height $+\beta_4 \times$ weight $+\epsilon$, where β_0 is the constant term; β_1, β_2, β_3, and β_4 are the regression coefficients for each variable, and ϵ represents the error term. Second, correlation analysis was performed. Pearson's correlation coefficient was used to assess the linear correlation between spleen volume and covariates such as age, height, and weight. Pearson's correlation coefficient ranges from -1 to 1, where values close to 1 indicate a strong positive correlation, values close to -1 indicate a strong negative correlation, and values close to 0 indicate no correlation.

The differences in MICB, ULBP1 and splenic volume between the patient group and the normal group, as well as between the study group and the control group, were analyzed by independent sample *t-tests*. Paired sample *t-tests* were used to compare the values of MICB, ULBP1, and splenic volume before and after treatment within the study group and the control group. Additionally, the correlations between

symptom scores, plasma MICB levels, and splenic volume were assessed. $P < 0.05$ was considered statistically significant.

Results

Demographic and clinical data

A total of 160 participants were recruited, including 80 in the normal group (30 males and 50 females) and 80 in the patient group (36 males and 44 females). The patients were further randomly divided into two groups: the (S)-ketamine combined with antidepressant group (study group, $n = 40$; 18 males and 22 females) and the saline combined with antidepressant group (control group, $n = 40$; 18 males and 22 females). There were no significant differences in gender ($\chi^2 = 0.925$, $P = 0.335$), age ($t = -0.587$, $P = 0.558$), or education time ($t = -0.957$, $P = 0.158$) between the patient group and the normal group. Similarly, no significant differences were observed in age ($t = 1.048$, $P = 0.298$) and education time ($t = -1.084$, $P = 0.282$) between the study group and the control group. (See Table 1).

Effect of (S)-ketamine

After 24 h of administration, 10 patients in the study group showed complete remission (MADRS ≤ 10), another 10 patients showed effective remission (MADRS score reduction $\geq 30\%$), 18 patients showed partial remission, and 2 patients showed no remission. These results are consistent with previous studies [29, 40]. Additionally, at 28 days after administration, the overall clinical symptoms of depression in the study group were milder than those in the control group, as reflected by the MADRS score ($P < 0.001$). See Tables 2 and 3, and Fig. 2 for MADRS scores at different time points and Two-Way Repeated Measures ANOVA results.

Comparison of MICB, ULBP, and spleen volume between the patient group and the normal group at baseline

At baseline, the patient group had significantly higher levels of MICB than that in the normal group ($t = 29.837$, $P < 0.001$). No significant difference was observed in ULBP1 (ng/ml) ($P > 0.05$). The splenic volume in the patient group was significantly larger than that in the normal group ($t = 3.024$, $P = 0.003$). ANCOVA showed that age, height and weight had no significant effect on spleen volume ($P > 0.05$). (See Table 4).

Changes in MICB and ULBP1

At baseline, no significant difference was observed in MICB levels between the study group and the control group ($P > 0.05$). At 28 days after treatment, the MICB level in the study group was significantly lower than that in the control group ($t = -5.700$, $P < 0.001$). Additionally, MICB levels in the study group decreased significantly from baseline ($t = 6.742$, $P < 0.001$), while that in the control group decreased slightly, but not significantly ($P > 0.05$).

There was no difference in ULBP1 between the study group and the control group at baseline or at 28 days after treatment ($P > 0.05$). However, it was significantly lower than that at baseline in the study group ($t = 4.201$, $P < 0.001$), while it decreased slightly, but not significantly in the control group ($P > 0.05$) (See Table 5).

Changes in splenic volumes

At baseline, no significant difference in splenic volume was observed between the study group and the control group. At 4 weeks after treatment, splenic volume in the study group decreased significantly compared with that at baseline ($t = 2.729$, $P = 0.009$), while that in the control group decreased slightly but not significantly ($P > 0.05$). Additionally, no significant difference in splenic volume

Table 1 Comprehensive contrast of demographic and clinical data

Indexes	Patient Group ($n = 80$)		Normal Group ($n = 80$)	t/χ^2	P
	Study Group ($n = 40$)	Control Group ($n = 40$)			
Male/Female	18/22	18/22	30/50	0.925[a,b]	0.335
Age (years)	37.92 ± 11.74		39.00 ± 11.44	-0.587	0.558
	39.30 ± 10.69	36.55 ± 12.69	-	1.048	0.298
Education time (years)	14.11 ± 2.58		14.51 ± 2.71	-0.957	0.158
	13.80 ± 2.67	14.43 ± 2.48	-	1.084	0.282

Note: the patient group = the MDD group; both the study group and the control group were included in the MDD group, with (S)-ketamine as the intervention factor

[a] χ^2-test

[b] Contrasting the patient group with the normal control group

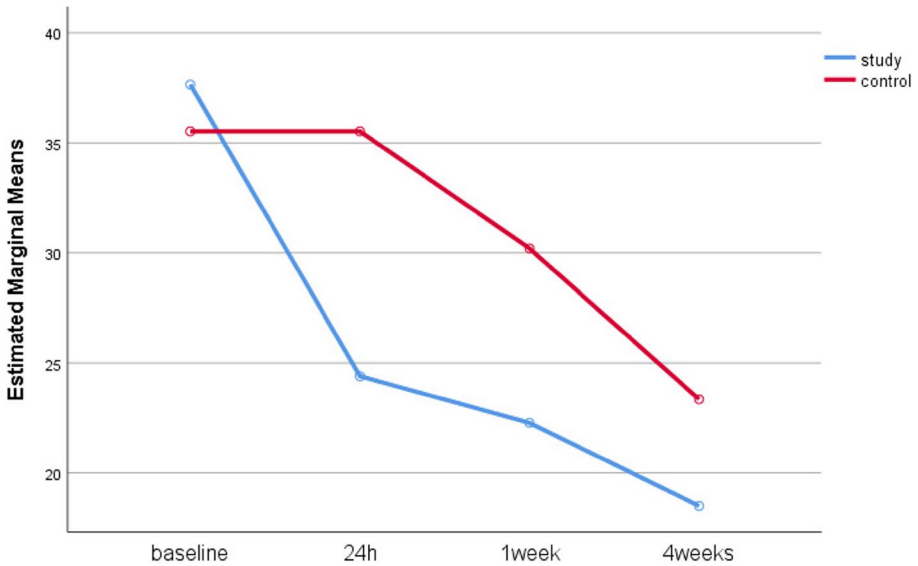

Fig. 2 Trend of Marginal Means of MADRS scores in the study group [(S)-ketamine] and the control group (saline). The figure illustrates that both the study group and the control group exhibited a decreasing trend in scores following treatment. Specifically, the study group had lower scores than the control group at 24 h, 1 weekend, and 4 weekends, with the most significant difference observed at 24 h

Table 2 MADRS scores of the study group and the control group at different time points

Group	Time			
	Baseline	**24 h**	**1 week**	**4 weeks**
Study Group ($n=40$)	37.65 ± 5.65	24.40 ± 11.83	22.28 ± 11.0.3	18.50 ± 10.35
Control Group ($n=40$)	35.53 ± 7.33	35.53 ± 7.33	30.20 ± 6.75	23.35 ± 6.63

Note: *MADRS* Montgomery–Åsberg Depression Rating Scale; both the study group and the control group were included in the MDD group, with (S)-ketamine as the intervention factor

Table 3 MADRS scores multivariate test results

Effect	Value[a]	Hypothesis df	Partial η^2	*F*	*P*
Time	*0.874*	*3.000*	*0.874*	*175.64*	*< 0.001*
Time × Group	*0.503*	*3.000*	*0.503*	*25.69*	*< 0.001*

The Mauchly test result (*W=0.307, P<0.001*) indicated that the data violated the sphericity assumption and exhibited asymmetry. Therefore, a multivariate test was employed. The Bonferroni-adjusted test results revealed a significant time effect on MADRS scores (*F=175.64, P<0.001*). The *partial* η^2 *value of 0.874* suggested that time had a substantial impact on the variation in the patients' MADRS scores. The interaction effect results (time point × group) (*F=25.69, P<0.001*) demonstrated that the changes in symptom scores over time differed between the study group and the control group. The *partial* η^2 *value of 0.503* indicated that group assignment significantly influenced the changes in the patients' MADRS scores. In other words, different treatment methods had a significant impact on symptom scores

Note: *MADRS* Montgomery–Åsberg Depression Rating Scale; both the study group and the control group were included in the MDD group, with (S)-ketamine as the intervention factor

[a] Pillai's Trace

was observed between the study group and the control group at 4 weeks ($P>0.05$) (See Table 5).

Correlation analysis

Correlation analysis revealed a positive correlation between MICB and splenic volume in the patient group ($r=0.276$, $P=0.013$) (See Fig. 3).

Discussion

The main findings of this study are as follows: (1) The patient group had significantly higher splenic volume and MICB levels than those in the normal group. Additionally, a positive correlation was observed between MICB and splenic volume in the patient group. (2) After (S)-ketamine or saline administration, the splenic volume was significantly smaller than that at baseline in the study

Table 4 Comparison of MICB, UBLP1, and splenic volume between the patient group and the normal group at baseline

Indexes	Patient Group ($n=80$)	Normal Group ($n=80$)	*t*	*P*
MICB (pg/ml)	164.94 ± 16.47	99.73 ± 10.52	*29.837*	*< 0.001*
UBLP1 (ng/ml)	115.23 ± 6.32	116.36 ± 75	*- 0.343*	*0.732*
Splenic volume (cm^3)	199.02 ± 68.81	170.50 ± 48.78	*3.024*	*0.003*

Note: the patient group = the MDD group

Table 5 Changes of MICB, UBLP1, and splenic volume before and after (S)-ketamine administration between the study group and the control group

Indexes		Baseline	4 weeks	*t*	*P*
MICB (pg/ml)	Study Group ($n=40$)	166.93 ± 12.17	138.63 ± 26.15	*6.742*	*< 0.001*
	Control Group ($n=40$)	162.95 ± 19.83	163.68 ± 9.43	*- 0.276*	*0.784*
UBLP1 (ng/ml)	Study Group ($n=40$)	115.91 ± 6.48	110.26 ± 8.60	*4.201*	*< 0.001*
	Control Group ($n=40$)	114.55 ± 6.17	112.50 ± 9.21	*1.108*	*0.275*
Splenic volume (cm^3)	Study Group ($n=40$)	198.04 ± 71.40	173.83 ± 55.32	*2.729*	*0.009*
	Control Group ($n=40$)	200.01 ± 67.03	194.52 ± 63.48	*0.669*	*0.508*

Note: Both the study group and the control group were included in the MDD group, with (S)-ketamine as the intervention factor; the unit of splenic volume is cubic centimeter, cm^3

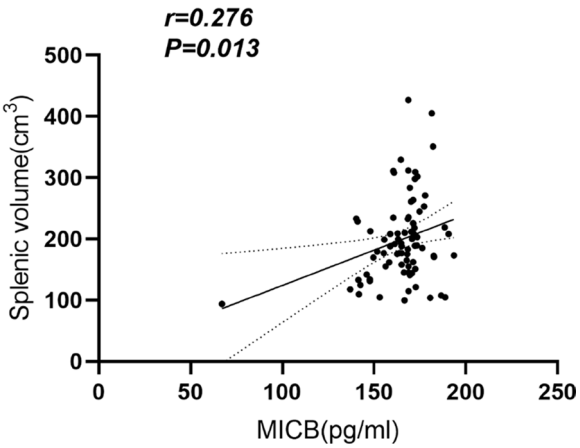

Fig. 3 Correlation between MICB and Splenic volume in the patient group (MDD) at baseline

group; in the control group, the splenic volume was only slightly but not significantly smaller. No significant difference was observed at 4 weeks. (3) After administration of (S)-ketamine or saline, the MICB and ULBP1 in the study group were significantly lower than those at baseline, while the MICB and ULBP1 decreased slightly but not significantly in the control group. A significant difference in MICB levels was observed between the study group and the control group at 4 weeks, but not in ULBP1.

Plasma MICB levels remain low in healthy individuals, but increase in many immune diseases and malignant tumors [16, 17, 22]. Some scholars have proposed that the level of NKG2D ligand-NKG2D may indicate the severity of inflammation in vivo [22]. Studies have shown that stress can activate NKG2D transcription in CSDS mice, and increase its levels in splenic cells, thereby altering spleen volume and weight. Mouse spleen NKG2D expression is positively correlated with spleen weight [15]. Another study has found that the number of splenic cells (mainly lymphocytes) in NKG2D-deficient mice decreases, compared with that in age-matched control mice, and NKG2D deficiency retards the development of NK cells [44]. These studies suggest that, in animal models, NKG2D may be involved in spleen function. Increased expression of NKG2D in the spleen may lead to depression-like phenotypes and increase spleen size and weight [15]. In this study, the plasma MICB level and the splenic volume in the patient group were significantly higher than those in the normal group, consistent with findings in animal studies. Notably, a positive correlation was observed between MICB and splenic volume ($r=0.276$, $P=0.013$), which is consistent with the findings of Zhang et al. [15]. Thus, we speculate that MICB may be dysregulated in the progression of MDD, and the MICB-NKG2D pathway may increase immune cells in the spleen, resulting in an enlargement in the splenic volume.

At 4 weeks after (S)-ketamine administration, MICB levels in the study group decreased significantly from baseline, but only slightly in the control group. The MICB in the study group was significantly lower than that in the control group ($P<0.001$). Previous studies

have shown that ketamine has anti-inflammatory effects. Subanesthetic doses of ketamine (10 mg/kg) effectively reduce circulating classical proinflammatory monocytes and increase the expression of other M2 macrophage subtypes in the spleen and central nervous system [45]. Animal studies have also shown that ketamine has anti-NKG2D properties, making ketamine a potential drug for treating certain inflammation-related diseases, such as Crohn's disease and rheumatoid arthritis [15]. Persistent immune injury may also be related to the inefficacy of antidepressants for refractory or recurrent depression [46]. Combined with the previous finding that ketamine can reverse the increase of NKG2D in mice [15], the present study suggests that (S)-ketamine may repress MICB expression and reduce, at least in part, the inflammatory response in MDD patients. Another result of this study supports this suggestion: compared with that at baseline, the plasma ULBP1 level did not change in the control group at 4 weeks, while that in the study group decreased significantly. This indicates that (S)-ketamine may have ameliorated the inflammatory response in the study group. The harmful effects of stress may be attributed to its immunosuppressive characteristics [47]. Some animal studies have shown that reducing inflammation mitigates the harmful effects of stress [48, 49]. This may explain why depressive symptoms in the study group were generally milder than those in the control group at 4 weeks, and also suggests that antidepressant mechanism of (S)-ketamine may be partially achieved by repressing inflammation in the body.

Over the past two decades, psychiatric studies have provided support for the hypothesis that inflammatory processes and brain-immune interactions are involved in the pathogenesis of major depression, and that inflammation may lead to serotonin and noradrenergic dysfunction. Stimuli, such as inflammation and chronic stress, can trigger the activation of brain immune cells called microglia, which release pro-inflammatory cytokines. These cytokines can lead to major depression and neurodegeneration by activating the hypothalamus-pituitary adrenal axis and increasing the activity of indoleamine 2,3-dioxygenase (IDO) [11]. The spleen connects the immune system to the central nervous system through the brain-spleen axis. The interaction between the splenic autonomic nervous system and immune cells is dynamic, maintaining the balance of the neuroimmune system [14]. When this balance is broken, the neural network may lose its stability, leading to the activation of microglia and ultimately neuroinflammation [11]. Studies have shown that in the Alzheimer's disease model, the spleen structure of mice is destroyed and severely enlarged, accompanied by a decrease in B lymphocytes [50], and a significant increase in chemokine receptors in spleen tissue [51]. A study has identified that 53 genes are differentially expressed in the spleen of depression rats (including 11 enriched in amino acid biosynthesis in the spleen), and the percentage of lymphocytes increase significantly [52]. Additionally, the expression of colony-stimulating factor 1 receptor and transcription factor PU.1 in spleen tissue of MDD patients is altered [53]. These findings indicate the spleen may play a role in serious mental disorders, such as MDD [14]. Studies have shown that lipopolysaccharide can increase expression of genes in the heme biosynthesis II pathway in mice, thus eliciting e depressive-like behavior in mice and changing spleen weight or plasma proinflammatory cytokine levels. The used of ketamine can block the above processes, reverse the increase in spleen weight, eliminate inflammation, and produce a lasting antidepressant effect [54]. This suggests that improving spleen function may help relieve depressive symptoms [47]. In this study, the splenic volume in the study group at 4 weeks was significantly smaller than that at baseline, suggesting inflammation was resolved and spleen function was restored following the administration of S-ketamine.

It was noteworthy that at 4 weeks, the spleen volume in the control group also showed a downward trend, although this change was not statistically significant compared with that at baseline. This trend may have led to the lack of statistical difference in spleen volume between the study group and the control group at 4 weeks. There are many reasons for the enlargement of splenic volume. In most cases, splenomegaly is not caused by pathological changes of the spleen itself, but by complications of other systemic diseases. The physiology in the human body is far more complex than that of experimental animals. In this study, due to the limitations of clinical research conditions, we could not control the subjects'diet, medication, etc., which is also a limitation of this study. This may lead to other factors affecting spleen morphology. For example, drugs (including antidepressants) can interfere with the size and function of the spleen [55]. Therefore, the relationship between the spleen and depression remains to be further studied.

Conclusion

The splenic abnormalities may be involved in the pathogenesis of MDD via the MICB-NKG2D pathway. In addition to its rapid antidepressant effect, (S)-ketamine can also attenuate inflammatory responses and enhance splenic function to promote short-term recovery in patients.

Acknowledgements

The authors thank Yongke Cao from Nanjing Medical University for professional editing of this article.

Authors' contributions
GQ Wang and K Zhang conceptualized the study. ZQ Lin performed the experiments. ZQ Lin and GQ Wang had access to all data and take responsibility for the accuracy of the data analysis. ZQ Lin performed the data analysis and interpreted the results. TL Wang, LM Cao, ZhQ Wang and XY Xu discussed with all authors about the data. All authors contributed to and approve of the final manuscript.

Funding
This study was supported by the research project of the Wuxi Health Commission on the MICB-NKG2D signaling pathway and the antidepressant mechanism of (S)-ketamine (M202010).

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Wuxi Mental Health Center (Batch Number: WXMHCIRB2020LLky039). All participants provided written informed consent.

Consent for publication
All authors have approved the manuscript for publication.

Competing interests
The authors declare no competing interests.

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Received: 8 February 2024 Accepted: 14 April 2025
Published online: 12 May 2025

References

1. Dantzer R, Cohen S, Russo SJ, Dinan TG. Resilience and immunity. Brain Behav Immun. 2018;74:28–42.
2. Godbout JP, Moreau M, Lestage J, Chen J, Sparkman NL, O'Connor J, et al. Aging exacerbates depressive-like behavior in mice in response to activation of the peripheral innate immune system. Neuropsychopharmacology. 2008;33(10):2341–51.
3. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry. 2009;65(9):732–41.
4. Elomaa A-P, Niskanen L, Herzig K-H, Viinamäki H, Hintikka J, Koivumaa-Honkanen H, et al. Elevated levels of serum IL-5 are associated with an increased likelihood of major depressive disorder. BMC Psychiatry. 2012;12:2.
5. Kim YK, Na KS, Myint AM, Leonard BE. The role of pro-inflammatory cytokines in neuroinflammation, neurogenesis and the neuroendocrine system in major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2016;64:277–84.
6. Frere JJ, Serafini RA, Pryce KD, Zazhytska M, Oishi K, Golynker I, et al. SARS-CoV-2 infection in hamsters and humans results in lasting and unique systemic perturbations after recovery. Sci Transl Med. 2022;14(664):eabq3059.
7. Capuron L, Miller AH. Cytokines and psychopathology: lessons from interferon-alpha. Biol Psychiatry. 2004;56(11):819–24.
8. Irwin M. Psychoneuroimmunology of depression: clinical implications. Brain Behav Immun. 2002;16(1):1–16.
9. Al-Hakeim HK, Al-Rammahi DA, Al-Dujaili AH. IL-6, IL-18, sIL-2R, and TNFalpha proinflammatory markers in depression and schizophrenia patients who are free of overt inflammation. J Affect Disord. 2015;182:106–14.
10. Carboni L, McCarthy DJ, Delafont B, Filosi M, Ivanchenko E, Ratti E, et al. Biomarkers for response in major depression: comparing paroxetine and venlafaxine from two randomised placebo-controlled clinical studies. Transl Psychiatry. 2019;9(1):182.
11. Mok SW, Wong VK, Lo HH, de Seabra Rodrigues Dias IR, Leung EL, Law BY, et al. Natural products-based polypharmacological modulation of the peripheral immune system for the treatment of neuropsychiatric disorders. Pharmacol Ther. 2020;208:107480.
12. Wohleb ES, McKim DB, Shea DT, Powell ND, Tarr AJ, Sheridan JF, et al. Re-establishment of anxiety in stress-sensitized mice is caused by monocyte trafficking from the spleen to the brain. Biol Psychiatry. 2014;75(12):970–81.
13. You Z, Luo C, Zhang W, Chen Y, He J, Zhao Q, et al. Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: involvement in depression. Behav Brain Res. 2011;225(1):135–41.
14. Hashimoto K. Molecular mechanisms of the rapid-acting and long-lasting antidepressant actions of (R)-ketamine. Biochem Pharmacol. 2020;177:113935.
15. Zhang K, Sakamoto A, Chang L, Qu Y, Wang S, Pu Y, et al. Splenic NKG2D confers resilience versus susceptibility in mice after chronic social defeat stress: beneficial effects of (R)-ketamine. Eur Arch Psychiatry Clin Neurosci. 2021;271(3):447–56.
16. Vadstrup K, Galsgaard ED, Jensen H, Lanier LL, Ryan JC, Chen SY, et al. NKG2D ligand expression in Crohn's disease and NKG2D-dependent stimulation of CD8(+) T cell migration. Exp Mol Pathol. 2017;103(1):56–70.
17. Ivanova M, Al Hadra B, Yordanov S, Lesichkova S, Stoyanov H, Shivarov V, et al. Associations of high-resolution-typing-defined MICA and MICB polymorphisms, and the levels of soluble MICA and MICB with Oral Squamous Cell Carcinoma in Bulgarian patients. J Oral Pathol Med. 2021;50(8):758–65.
18. Gonzalez S, Lopez-Soto A, Suarez-Alvarez B, Lopez-Vazquez A, Lopez-Larrea C. NKG2D ligands: key targets of the immune response. Trends Immunol. 2008;29(8):397–403.
19. Zingoni A, Molfetta R, Fionda C, Soriani A, Paolini R, Cippitelli M, et al. NKG2D and Its Ligands: "One for All, All for One." Front Immunol. 2018;9:476.
20. Babic M, Romagnani C. The Role of Natural Killer Group 2, Member D in Chronic Inflammation and Autoimmunity. Front Immunol. 2018;9:1219.
21. Raulet DH, Gasser S, Gowen BG, Deng W, Jung H. Regulation of ligands for the NKG2D activating receptor. Annu Rev Immunol. 2013;31:413–41.
22. Babic M, Dimitropoulos C, Hammer Q, Stehle C, Heinrich F, Sarsenbayeva A, et al. NK cell receptor NKG2D enforces proinflammatory features and pathogenicity of Th1 and Th17 cells. J Exp Med. 2020;217(8):e20190133.
23. Cox ST, Hernandez D, Danby R, Turner TR, Madrigal JA. Diversity and characterisation of polymorphic 3' untranslated region haplotypes of MICA and MICB genes. HLA. 2018;92(6):392–402.
24. Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, Perez AM, et al. Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. Am J Psychiatry. 2013;170(10):1134–42.
25. Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, et al. Antidepressant effects of ketamine in depressed patients. Biol Psychiatry. 2000;47(4):351–4.
26. Murrough JW, Perez AM, Pillemer S, Stern J, Parides MK, Aan het Rot M, et al. Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. Biol Psychiatry. 2013;74(4):250–6.
27. Maeng S, Zarate CA, Du J, Schloesser RJ, McCammon J, Chen G, et al. Cellular Mechanisms Underlying the Antidepressant Effects of Ketamine: Role of α-Amino-3-Hydroxy-5-Methylisoxazole-4-Propionic Acid Receptors. Biol Psychiat. 2008;63(4):349–52.
28. Xu Y, Hackett M, Carter G, Loo C, Gálvez V, Glozier N, et al. Effects of Low-Dose and Very Low-Dose Ketamine among Patients with Major Depression: a Systematic Review and Meta-Analysis. Int J Neuropsychopharmacol. 2016;19(4).
29. Correia-Melo FS, Leal GC, Vieira F, Jesus-Nunes AP, Mello RP, Magnavita G, et al. Efficacy and safety of adjunctive therapy using esketamine or racemic ketamine for adult treatment-resistant depression: A randomized, double-blind, non-inferiority study. J Affect Disord. 2020;264:527–34.

30. Sumner RL, McMillan RL, Forsyth A, Muthukumaraswamy SD, Shaw AD. Neurophysiological evidence that frontoparietal connectivity and GABA-A receptor changes underpin the antidepressant response to ketamine. Transl Psychiatry. 2024;14(1):116.
31. Carlson PJ, Diazgranados N, Nugent AC, Ibrahim L, Luckenbaugh DA, Brutsche N, et al. Neural Correlates of Rapid Antidepressant Response to Ketamine in Treatment-Resistant Unipolar Depression: A Preliminary Positron Emission Tomography Study. Biol Psychiat. 2013;73(12):1213–21.
32. Belujon P, Grace AA. Restoring Mood Balance in Depression: Ketamine Reverses Deficit in Dopamine-Dependent Synaptic Plasticity. Biol Psychiat. 2014;76(12):927–36.
33. Chen H, Zhao X, Ma X, Ma H, Zhou C, Zhang Y, et al. Effects of esketamine and fluoxetine on depression-like behaviors in chronic variable stress: a role of plasma inflammatory factors. Front Psychiatry. 2024;15:1388946.
34. Quintanilla B, Zarate CA Jr, Pillai A. Ketamine's mechanism of action with an emphasis on neuroimmune regulation: can the complement system complement ketamine's antidepressant effects? Mol Psychiatry. 2024;29(9):2849–58.
35. Fateh SM, Mohammed NA, Mahmood KA, Hasan AH, Tahir SH, Kakamad FH, et al. Sonographic measurement of splenic size and its correlation with body parameters. Med Int. 2023;3(1):7.
36. Özdikici M. The relationship between splenic length in healthy children from the Eastern Anatolia Region and sex, age, body height and weight. Journal of ultrasonography. 2018;18(72):5–8.
37. Okazaki M, Tominaga K, Higuchi H, Utagawa I, Nakamura E, Noguchi M, et al. Predictors of response to electroconvulsive therapy obtained using the three-factor structure of the Montgomery and Asberg Depression Rating Scale for treatment-resistant depressed patients. J ect. 2010;26(2):87–90.
38. Rodrigues NB, McIntyre RS, Lipsitz O, Lee Y, Cha DS, Shekotikhina M, et al. A simplified 6-Item clinician administered dissociative symptom scale (CADSS-6) for monitoring dissociative effects of sub-anesthetic ketamine infusions. J Affect Disord. 2021;282:160–4.
39. Maust D, Cristancho M, Gray L, Rushing S, Tjoa C, Thase ME. Psychiatric rating scales. Handb Clin Neurol. 2012;106:227–37.
40. Singh JB, Fedgchin M, Daly E, Xi L, Melman C, De Bruecker G, et al. Intravenous Esketamine in Adult Treatment-Resistant Depression: A Double-Blind, Double-Randomization. Placebo-Controlled Study Biol Psychiatry. 2016;80(6):424–31.
41. Salih HR, Antropius H, Gieseke F, Lutz SZ, Kanz L, Rammensee HG, et al. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood. 2003;102(4):1389–96.
42. Desimio MG, Finocchi A, Di Matteo G, Di Cesare S, Giancotta C, Conti F, et al. Altered NK-cell compartment and dysfunctional NKG2D/NKG2D-ligand axis in patients with ataxia-telangiectasia. Clin Immunol. 2021;230:108802.
43. Yoo J, Kim SW, Lee DH, Bae JS, Cho EJ. Prognostic role of spleen volume measurement using computed tomography in patients with compensated chronic liver disease from hepatitis B viral infection. Eur Radiol. 2021;31(3):1432–42.
44. Zafirova B, Mandaric S, Antulov R, Krmpotic A, Jonsson H, Yokoyama WM, et al. Altered NK cell development and enhanced NK cell-mediated resistance to mouse cytomegalovirus in NKG2D-deficient mice. Immunity. 2009;31(2):270–82.
45. Nowak W, Grendas LN, Sanmarco LM, Estecho IG, Arena AR, Eberhardt N, et al. Pro-inflammatory monocyte profile in patients with major depressive disorder and suicide behaviour and how ketamine induces anti-inflammatory M2 macrophages by NMDAR and mTOR. EBioMedicine. 2019;50:290–305.
46. Bortolato B, Carvalho AF, Soczynsk JK, Perini GI, McIntyre RS. The involvement of TNF-α in cognitive dysfunction associated with major depressive disorder: An opportunity for domain specific treatments. Curr Neuropharmacol. 2015;13(05):558–76.
47. Elenkov IJ. Glucocorticoids and the Th1/Th2 balance. Ann N Y Acad Sci. 2004;1024:138–46.
48. Myint AM, Steinbusch HW, Goeghegan L, Luchtman D, Kim YK, Leonard BE. Effect of the COX-2 inhibitor celecoxib on behavioural and immune changes in an olfactory bulbectomised rat model of depression. Neuro-ImmunoModulation. 2007;14(2):65–71.
49. Guo JY, Li CY, Ruan YP, Sun M, Qi XL, Zhao BS, et al. Chronic treatment with celecoxib reverses chronic unpredictable stress-induced depressive-like behavior via reducing cyclooxygenase-2 expression in rat brain. Eur J Pharmacol. 2009;612(1–3):54–60.
50. Yang S-H, Kim J, Lee MJ, Kim Y. Abnormalities of plasma cytokines and spleen in senile APP/PS1/Tau transgenic mouse model. Scient Rep. 2015;5(1):15703.
51. Subramanian S, Ayala P, Wadsworth TL, Harris CJ, Vandenbark AA, Quinn JF, et al. CCR6: A biomarker for Alzheimer's-like disease in a triple transgenic mouse model. J Alzheimers Dis. 2010;22(2):619–29.
52. Zhan H, Huang F, Yan F, Zhao Z, Zhang J, Cui T, et al. Alterations in splenic function and gene expression in mice with depressive-like behavior induced by exposure to corticosterone. Int J Mol Med. 2017;39(2):327–36.
53. Zhang J, Chang L, Pu Y, Hashimoto K. Abnormal expression of colony stimulating factor 1 receptor (CSF1R) and transcription factor PU.1 (SPI1) in the spleen from patients with major psychiatric disorders: a role of brain-spleen axis. J Affect Disord. 2020;272:110–5.
54. Ma L, Wang L, Qu Y, Wan X, Hashimoto K. A role of splenic heme biosynthesis pathway in the persistent prophylactic actions of arketamine in lipopolysaccharide-treated mice. Transl Psychiatry. 2023;13(1):269.
55. Petroianu A. Drug-induced splenic enlargement. Expert Opin Drug Saf. 2007;06(02):199–206.

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