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Research report

Altered expression of $CD4^+CD25^+$ regulatory T cells and its 5-HT_{1a} receptor in patients with major depression disorder

Yi Li, Bo Xiao, Wenjing Qiu, Le Yang, Bo Hu, Xiaolin Tian, Huan Yang *

Department of Neurology, Xiangya Hospital, Central South University, 87 Xiang Ya Road, Changsha, Hunan, 410008, China

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ABSTRACT

Background: There is growing interest in immune imbalance observed in major depression, but the mechanism of this imbalance is still not well understood. $CD4^+CD25^+$ regulatory T (Treg) cells are known to play an important role in immune regulation. However, their role in major depression has rarely been explored. The aims of this study are to determine the immune imbalance status in patients when first diagnosed with major depression, and to explore possible indications of Treg cell involvement in immune imbalance of major depression.
Methods: 27 patients with major depression and 27 healthy individuals were recruited in the study. The immune imbalance was observed by the analysis of Th cell cytokines and $CD4^+CD25^+$ Treg cell level. The 5-HT in peripheral serum was measured with HPLC, and changes of Foxp3 and 5-HT_{1a} receptor were observed by RT-PCR and double-immunofluorescence.
Results: Despite the similar total lymphocyte count between patients and healthy individuals, the Th1/Th2 ratio increased and the $CD4^+CD25^+$ Treg cells decreased in patients' peripheral blood. Moreover, we found that the level of 5-HT was decreased in peripheral serum, and the expression of 5-HT_{1a} receptor was also decreased in peripheral lymphocytes of major depression patients, notably in the $CD4^+CD25^+$ Treg cells rather than $CD4^+CD25^-$ T cells.
Limitations: The details of interaction between 5-HT_{1a} receptors and Treg cells in major depression need further exploration.
Conclusion: $CD4^+CD25^+$ Treg cells may contribute to the immune imbalance in patients with major depression, which could involve its interaction with 5-HT system.

1. Introduction

Nowadays major depression is recognized not only as one of the major psychiatric diseases, but also as an immune system disorder (Benton et al., 2007; Irwin and Miller, 2007). Dysregulation of the immune system has been observed in patients of major depression (Marazitti et al., 1992; Muller et al., 1993; Myint et al., 2005; Irwin and Miller, 2007; Miller et al., 2009). These patients exhibited unbalanced peripheral blood inflammatory biomarkers, such as augmented expression of IL-1, IL-6, IL-2, IL-12, soluble IL-6R, soluble IL-2R, and IFN-γ (Myint et al., 2005; Kim et al., 2002; Maes et al., 1993, 1995; Dimopoulos et al., 2008; Kubera et al., 2001), although some conflicting results were also reported (Frank et al., 2001; Rothermundt et al., 2001; Brambilla et al., 2004). These changes have been considered in terms of the imbalance between pro- and anti-inflammatory cytokines, referred to as Th1/Th2 cells. However, the mechanism of the immune imbalance of major depression is still unclear.

One of the major theories of depression pathogenesis is the monoamine hypothesis: low levels of brain monoamine neurotransmitters, specifically 5-hydroxytryptamine (5-HT), could induce depression (Rothermundt et al., 2001). Besides being a neurotransmitter in the central nervous system, 5-HT is also an immune modulator in the periphery. It has been shown to influence the function of NK cells, macrophages, T cells and pre-B lymphocytes (Frank et al., 2001; Suguro et al., 2006; Abdouh et al., 2004). The immune regulatory activities of 5-HT are mediated through several different receptors.

* Corresponding author. Tel.: +86 731 4327236; fax: +86 731 4327401.
E-mail address: yangh69@yahoo.com (H. Yang).

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5-HT_{1a} receptor (5-HT_{1a}R) is not only one of the most abundantly expressed 5-HT receptor subtypes in the brain (Albert and Lemonde, 2004), whose important role in major depression has been explored in both patients and 5-HT_{1a}R knockout mice models (Ramboz et al., 1998; Drevets et al., 2007), but it is also related to activities of human T cells in the periphery (Aune et al., 1993).

$CD4^+CD25^+$ regulatory T (Treg) cells are a highly specialized T cell subpopulation that is pivotal in suppression of the immune response (Maloy and Powrie, 2001), whose generation requires the transcription factor Foxp3 (Hori et al., 2003). Both *in vitro* and *in vivo* analyses suggest that Treg cells could suppress the proliferation and/or cytokine production of T effector cells (Suri-Payer et al., 1998; Piccirillo and Shevach, 2001; Li et al., 2007). Its dysfunction is involved in the pathogenesis of many autoimmune diseases, such as systemic lupus erythematosus, multiple sclerosis, asthma, autoimmune rheumatic diseases etc. (Belkaid and Rouse, 2005; Baecher-Allan and Hafler, 2006). Treg cells can exert their suppression capacity by utilizing various mechanisms: it could be mediated by transforming growth factor-β_1 (TGF-β_1), interleukin-10 (IL-10) or other cytokines, which have been extensively studied in a variety of infections (Tang and Bluestone, 2008). However, the role of $CD4^+CD25^+$ Treg cells in major depression, which also exhibited immune imbalance, has not been explored yet.

The aims of the present study are to determine the immune imbalance status in patients when they are first diagnosed with major depression, to observe the changes of 5-HT and 5-HT_{1a}R in the peripheral lymphocytes, and to explore the alteration of Treg cells and the changes of 5-HT_{1a}R related to Treg cells in these patients.

2. Methods

2.1. Subjects

From March 1, 2006 to July 30, 2007, patients from Xiang Ya Hospital, Central South University, China, were considered if they were diagnosed with major depression for the first time and had not used antidepressant medications before. The diagnosis criteria we used are defined in the Diagnostic and Statistical Manual (DSM-IV), and the diagnosis was made with the Structured Clinical Interview process from DSM-IV. The patients were then screened to meet the criteria as follows: no concomitant psychiatric illness, no history of infection or other immune system disorders, no history of epilepsy or glaucoma, no liver or kidney related disorders, no history of alcohol dependence/abuse and drug addiction; no intake of alcohol or tobacco in the last 6 months, no immunomodulator medication, females were not in lactation or in gestational period. Twenty-seven patients were recruited for the present study. The psychopathological status of the patients was assessed by the Hamilton Depression Scale (HAMD)-24 and the scores were all higher than 18. Twenty-seven age- and gender-matched healthy individuals were recruited for the healthy control group, using the same screening criteria described above. All participants gave their informed written consent to participate.

Ulnar venous blood (23 ml) was withdrawn between 8 a.m. and 9 a.m. from each subject the day after the clinical assessment was finished. 20 ml venous blood from each patient was anti-coagulated with heparin and stored at 4 °C for lymphocytes isolation. Serum was separated from the remaining 3 ml venous blood and stored at −20 °C.

2.2. High-performance liquid chromatography (HPLC) analysis of 5-HT in peripheral serum

To test the concentration of 5-HT in the peripheral serum of both patients and healthy controls, the samples were analyzed by HPLC (LC-6A, Shimadzu Corporation, Japan) with a 250 mm × 4.6 mm C18 chromatographic column (particle size 5 μm). The mobile phase consisted of 20 mmol/l acetyltriethyl citrate (with 0.1 mmol/l Na–EDTA, pH = 4.50), methyl cyanide and methanol (90:3:7). The flow rate was set up as 1.0 mmol/min at 35 °C (voltage = 0.75 V, AUFS = 50 mV). The samples were detected with an external reference method and stock standard solution of 11.5 μg/ml 5-HT (Sigma, USA) was used to produce external standard curves.

2.3. Enzyme-linked immunosorbent assay (ELISA) analysis of IL-2, IL-10 and TGF-β_1

The concentrations of IL-2, IL-10 and TGF-β_1 in peripheral serum of patients and healthy controls were tested following the instructions of the human IL-2, IL-10, and TGF-β_1 ELISA kits (Jingmei Biological Techniques, China). Optical density (OD) of cytokines was read by a microplate reader at 450 nm (Perkin Elmer Company, USA) and the data was analyzed by CurveExpert 1.3 software.

2.4. Lymphocytes separation and regulatory T cells isolation

To isolate the peripheral blood lymphocytes, each collected 20 ml serum sample was first separated into 5 ml and 15 ml tubes, from which the isolated lymphocytes would be used for RT-PCR analysis and Treg cell isolation separately. Lymphocytes were then isolated with the Ficoll–Hypaque density gradient procedure. The lymphocytes collected for $CD4^+CD25^+$ Treg cell isolation were resuspended in RPMI 1640 medium with 0.5% BSA, and the concentrations were adjusted to 1×10^7/ml. Aseptic techniques were maintained throughout all steps.

$CD4^+CD25^+$ Treg cells were isolated with the Dynal $CD4^+CD25^+$ Treg immune beads Kit (DYNAL biotech/Invitrogen, Norway). $CD4^+$T cells were first indirectly selected from the total lymphocyte cells: the obtained lymphocytes were incubated with 50 μl CD4 Antibody Mix/50 μl CD45RA mAb/100 μl foetal calf serum (FCS) per 10^7/ml lymphocytes for 20 min; after washing with PBS/0.1%BSA, the cells were incubated with 1 ml Depletion Dynabeads per 10^7/ml lymphocytes for 30 min at RT. The rosettes formed after the incubation were gently suspended and the containing tube was placed on a Dynal magnetic particle concentrator (DYNAL biotech, Norway) for 5 min. The supernatant, which had the isolated $CD4^+$ T cells, was collected. The $CD4^+CD25^+$ T cells were then directly isolated from $CD4^+$ T cells by incubating the obtained $CD4^+$ T cells with 200 μl Dynabeads CD25 per 10^7/ml $CD4^+$ T cells for 30 min at 8 °C, and then placing the containing tube in the magnetic device for 2 min. Both the supernatant ($CD4^+CD25^-$ T cells), and the $CD4^+CD25^+$ T cells which originally rosettedly

attached to the tube wall and were later detached by using a DETACHaBEAD kit, were collected. The collected cells were resuspended in RPMI 1640 medium with 0.5% BSA, counted and conducted for immunofluorescence as described in Section 2.6.

2.5. Semiquantitative RT-PCR for 5-HT_{1a}R and Foxp3

Total RNA was isolated from lymphocytes of both patients and healthy controls with a reverse transcriptase kit with Trizol method (Toyobo, Japan) to obtain cDNA. Primer designed to recognize sequences specific for 5-HT_{1a}R, Foxp3 and housekeeping gene β-actin were as follows: 5-HT_{1a}R (411-bp product): sense: 5′-GCC GCG TGC GCT CAT CTC G-3′, antisense: 5′-GCG GCG CCA TCG TCA CCT T-3′; Foxp3 (152-bp product): sense: 5′-CCT TGA GGC TAT CCA GCG TA-3′, antisense: 5′-ACT CAG GTT GTG GCG GAT GG-3′; β-actin (259-bp product): sense: 5′-CCT TGA GGC TAT CCA GCG TA-3′, antisense: 5′-GTT CAC ACG GCA GGC ATA CT-3′. Thirty five PCR cycles were run at 94 °C (denaturation, 5 min), 61 °C (annealing, 1 min), and 72 °C (extension, 1 min). Intensity of the different bands was quantified by measuring the OD value with OneDscan computer software package (Scanalytics, Fairfax, VA). Primers for β-actin were run simultaneously with other primers as an internal control and all results were expressed as the percentages of β-actin OD value. The cDNA amplification was linear in an amplification range of 28–38 cycles. Controls that ran without reverse transcriptase yielded no PCR products.

2.6. Double-immunofluorescence labeling of 5-HT_{1a}R and Foxp3

For double-immunofluorescence labeling of 5-HT_{1a}R and Foxp3, $CD4^+CD25^-$ T cells and $CD4^+CD25^+$ Treg cells from patients and healthy controls were suspended separately in 4% paraformaldehyde/0.01 M PBS at RT for 10 min. After fixation, samples were washed twice, resuspended in 50 μl PBS and adhered onto polyY-L-lysine coated slides (Sigma, USA) for 30 min at RT. The sections were then incubated in 10% normal goat serum with 0.1% Triton X-100 for 30 min. After washing with PBS twice, sections were incubated in a 100 μl solution containing mouse anti-Foxp3 (1:150; e-Bioscience, USA) and rabbit anti-5-HT_{1a}R (1:200; Santa Cruz, USA) for 2 nights. After gentle rinsing, sections were incubated in a mixture of TRITC-conjugated goat anti-rabbit IgG (1:1000; Santa Cruz, USA) and FITC-conjugated goat anti-mouse IgG (1:1000; Santa Cruz, USA) at RT for 2 h. Following the removal of the secondary antiserum, the slides were coverslipped with antifade medium Prolong Gold. The results were observed with a Zeiss confocal laser scanning microscope.

2.7. Statistical analysis

Data in Tables 1–2 are described using the mean and standard deviation ($\bar{x} \pm s$). Statistical significance of the differences between the patient group and the healthy control group was established using the Student's *t*-test; $p<0.05$ was considered to be significant. Correlation data in Table 3 was computed using the bivariate Pearson correlation test. The statistical package used for the analysis was SPSS 11.5.

3. Results

3.1. Demographic data

The demographic data of the 54 study subjects are listed in Table 1. We recruited 27 patients diagnosed with major depression and 27 healthy individuals based on the screening criteria described previously. The patient group included 13 males and 14 females, ranging from 18 to 56 years of age (30.60 ± 2.18 years). The duration of their depression episode on admission was 17.16 ± 4.60 months, with 80% of them being a melancholic depression subtype. The mean value of the Hamilton Rating Scales was 21.38 ± 1.61. There was no significant difference in the male/female ratio between patient group and healthy control group ($p>0.05$).

3.2. Concentrations of 5-HT in peripheral serum

The concentration of the 5-HT in peripheral serum was measured with HPLC method for both patients and healthy controls. The level of peripheral 5-HT in healthy controls was 0.801 ± 0.760 μg/ml, while the level in the major depression patients was 0.249 ± 0.080 μg/ml, which showed an evident decrease compared to that of controls ($p<0.01$) (Fig. 1).

3.3. Cytokine levels of IL-2, IL-10 and TGF-β_1 in peripheral serum

The cytokine levels of IL-2, IL-10 and TGF-β_1, which represented the cytokines of the Th1, Th2 and Th3 respectively, were measured in the serum of patients and healthy individuals (Table 2). All three cytokines were detectable in each sample. The IL-2 level in major depression patients was 184.681 ± 8.472 ng/ml, showing approximately a two-fold increase compared to the levels of 82.845 ± 12.292 ng/ml in healthy controls ($p<0.05$). However, the IL-10 level in major depression patients showed a 29.48% decrease (6.765 ± 0.611 ng/ml) compared to that in the healthy control group (9.593 ± 0.921 ng/ml) ($p<0.01$). The level of TGF-β_1 was much lower than IL-2 or IL-10, and was detected in the pg/ml level. It showed 33.07% decrease in the patient group (14.042 ± 2.170 pg/ml) compared to the healthy control group (20.981 ± 3.980 pg/ml) ($p<0.01$). The ratio of Th1/Th2 cells as shown by the levels of IL-2/IL-10 had approximately a three-fold increase in depression patients (27.299 ± 2.973 compared to 8.635 ± 1.865 of healthy controls). There were no significant

Table 1
Demographic data of study subjects.

	Major depression	Healthy controls
Gender (male/female)	13/14	13/14
Age (years)	30.60 ± 2.18 (15–56)	31.13 ± 2.12 (22–55)
Duration of episode at admission (months)	17.16 ± 4.60	
Subtypes		
Melancholic depression	11/10	
Atypical depression	2/3	
Catatonic depression	0/1	
HDRS	21.38 ± 1.61	

HDRS: Hamilton Depression Rating Scale.

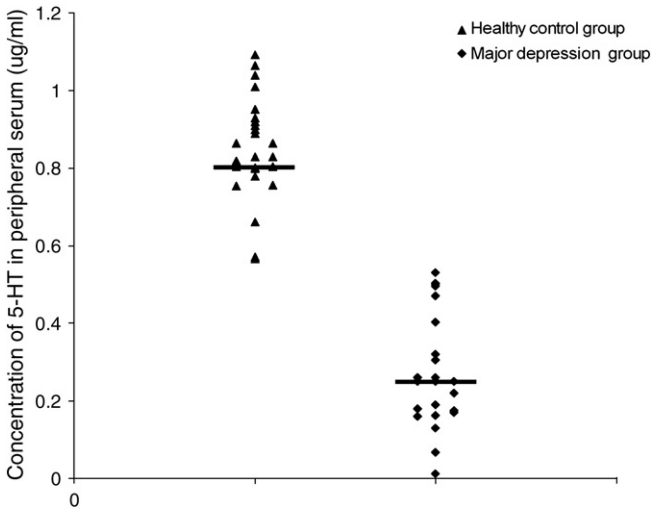

Fig. 1. Concentration of 5-HT in peripheral serum of major depression patients and healthy controls. The level of 5-HT was decreased in peripheral serum of the patient group (0.249 ± 0.080 μg/ml) compared to that in the healthy control group (0.801 ± 0.760 μg/ml).

differences of the cytokine levels between male and female (data not shown).

3.4. 5-HT_{1a}R and Foxp3 mRNA levels in peripheral blood lymphocytes

Expression of mRNA for 5-HT_{1a}R and Foxp3 was analyzed by RT-PCR (Fig. 2A–C). The levels were measured as its ratio to β-actin OD value (Fig. 2D). The level of 5-HT_{1a}R mRNA (0.376 ± 0.017) was significantly decreased in the patient group (Fig. 2B) compared to the level (0.462 ± 0.024) in the healthy control group (Fig. 2A). Foxp3 mRNA showed a similar decrease, since the level was 0.610 ± 0.097 in the patient group (Fig. 2C, 1–2) while the value in the healthy control group was 0.903 ± 0.117 (Fig. 2C, 3–4) ($p<0.01$).

3.5. $CD4^+CD25^+$ Treg population in peripheral blood lymphocytes

After isolating the lymphocytes, they were collected and counted. In the healthy control group, the level of lymphocytes was $3.797 \pm 0.756 \times 10^6$/ml, while in the patient group, the level of lymphocytes was $3.055 \pm 0.483 \times 10^6$/ml. There was no statistical difference between them ($p>0.05$). However, the level of $CD4^+CD25^+$ Treg cells in the patient group was significantly decreased ($7.834 \pm 0.252 \times 10^4$/ml) compared to the level in the healthy control group of $19.483 \pm 2.942 \times 10^4$/ml ($p<0.01$); and the percentages of $CD4^+CD25^+$ Treg cells in lymphocytes also showed similar decrease ($2.053 \pm 0.071\%$) compared to that of healthy controls ($4.792 \pm 0.714\%$) ($p<0.01$). These observations suggest that the immune imbalance of major depression was associated with dysregulation among different subgroups of lymphocytes, rather than the whole peripheral lymphocyte population (Fig. 3).

3.6. Expression of 5-HT_{1a}R and Foxp3 in $CD4^+CD25^+$ Treg cells

To explore the alteration of 5-HT_{1a}R correlated to $CD4^+CD25^+$ Treg cells, double-immunofluorescence for 5-HT_{1a}R and Foxp3 was performed. In the $CD4^+CD25^+$ Treg cells of the healthy control group, 5-HT_{1a}R was expressed most significantly on the membrane and less evident in the cytoplasm, by showing strong labeling at the outline of cells and moderate labeling in the cytoplasm; while Foxp3 labeling was expressed throughout the nuclei and cytoplasm (Fig. 4A–C). However, the expression of both 5-HT_{1a}R and Foxp3 was decreased in the $CD4^+CD25^+$ Treg cells of the patient group (Fig. 4D–F). The decrease of 5-HT_{1a}R appeared to be most prominent on the membrane of $CD4^+CD25^+$ Treg cells, since strong outline labeling could not be observed. Unlike the alterations in $CD4^+CD25^+$ Treg cells, 5-HT_{1a}R labeling did not show evident differences between patients and healthy controls in $CD4^+CD25^-$ T cells (Fig. 4G,I,J,L), both of which

Table 2
Concentrations of IL-2, IL-10 and TGF-β1 in peripheral serum.

	N	IL-2 (ng/ml)	IL-10 (ng/ml)	TGF-β_1 (pg/ml)	IL-2/IL-10
Healthy control	27	82.845 ± 12.292	9.593 ± 0.921	20.981 ± 3.980	8.635 ± 1.865
Major depression	27	184.681 ± 8.472 *	6.765 ± 0.611 **	14.042 ± 2.170 **	27.299 ± 2.973

* $p<0.05$.
** $p<0.01$.

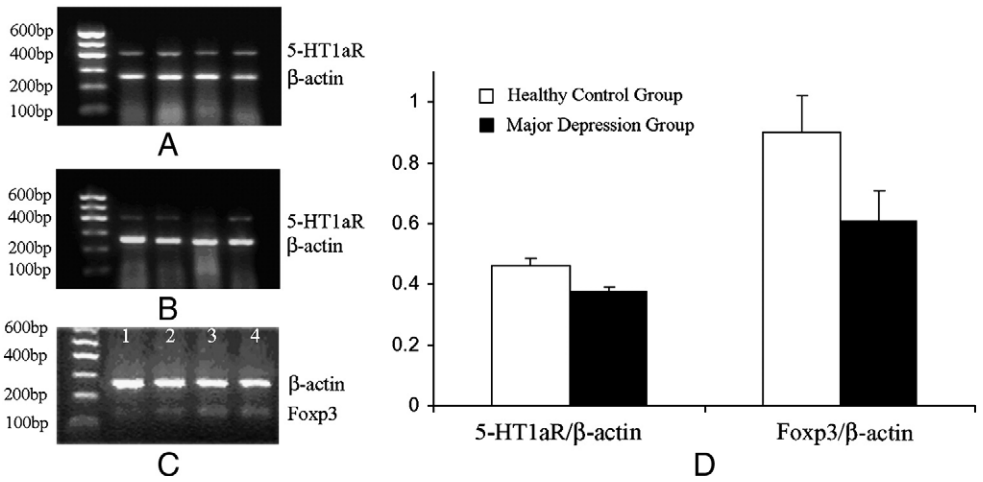

Fig. 2. Expression of mRNA for 5-HT_{1a}R and Foxp3 in major depression patients and healthy controls. (A) Expression of 5-HT_{1a}R mRNA in healthy controls. (B) Expression of Foxp3 mRNA in patients with major depression. (C) Expression of Foxp3 mRNA in patients with major depression (1,2) and healthy controls (3,4). (D) Comparison of mRNA levels for 5-HT_{1a}R or Foxp3 between patient group and healthy control group.

showed similar patterns as that in $CD4^+CD25^+$ Treg cells of the healthy control group. No Foxp3 labeling was observed in $CD4^+CD25^-$ T cells (Fig. 4H,K).

3.7. Correlation analysis of 5-HT, 5-HT_{1a}R mRNA, peripheral blood cytokine levels and $CD4^+CD25^+$ Treg cell population

We first conducted the Pearson correlation analysis between peripheral blood cytokine levels and $CD4^+CD25^+$ Treg cells to examine whether $CD4^+CD25^+$ Treg cells were correlated to Th cell levels in both major depression patients and healthy controls. We found that both IL-2 and TGF-β_1 were positively correlated with $CD4^+CD25^+$ Treg cell counts ($r=0.416$, $r=0.517$, respectively; $p<0.01$), the percentage of $CD4^+CD25^+$ Treg cell in peripheral lymphocytes ($r=0.446$, $r=0.588$, respectively; $p<0.01$), and Foxp3 mRNA expression ($r=0.307$, $p<0.05$; $r=0.449$, $p<0.01$; respectively). In addition, the IL-10 level was negatively correlated with Foxp3 mRNA expression ($r=-0.316$, $p<0.05$) (Table 3).

We also analyzed the correlation between 5-HT system and $CD4^+CD25^+$ Treg cells with the same method, to understand whether the 5-HT system possibly interacted with

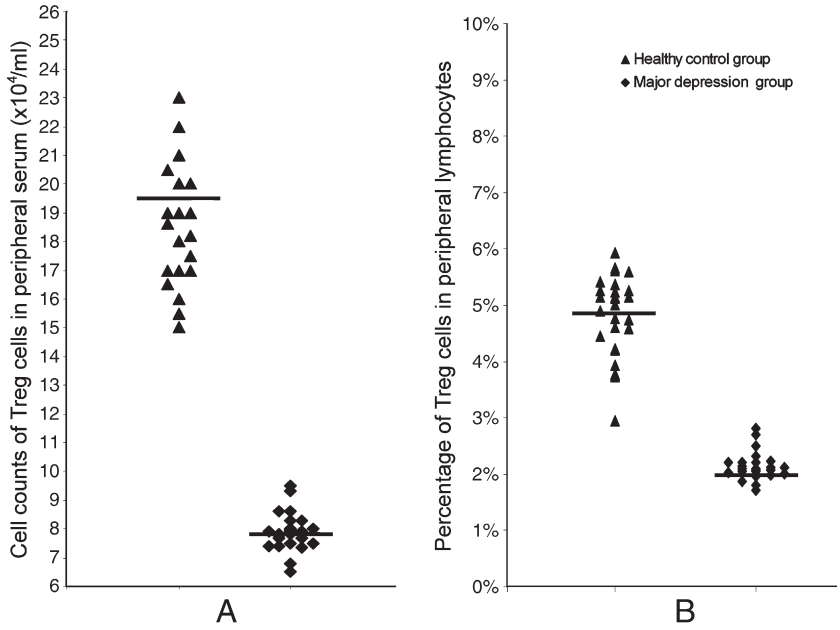

Fig. 3. The level of peripheral $CD4^+CD25^+$ Treg cells in major depression patients and healthy controls. (A) The cell count of $CD4^+CD25^+$ Treg cells showed prominent decrease in patients with major depression ($7.834\pm0.252\times10^4$/ml) compared to that in healthy controls ($19.483\pm2.942\times10^4$/ml). (B) The percentages of $CD4^+CD25^+$ Treg cells in lymphocytes also showed significant decrease in patients with major depression ($2.053\pm0.071\%$) compared to that in healthy controls ($4.792\pm0.714\%$).

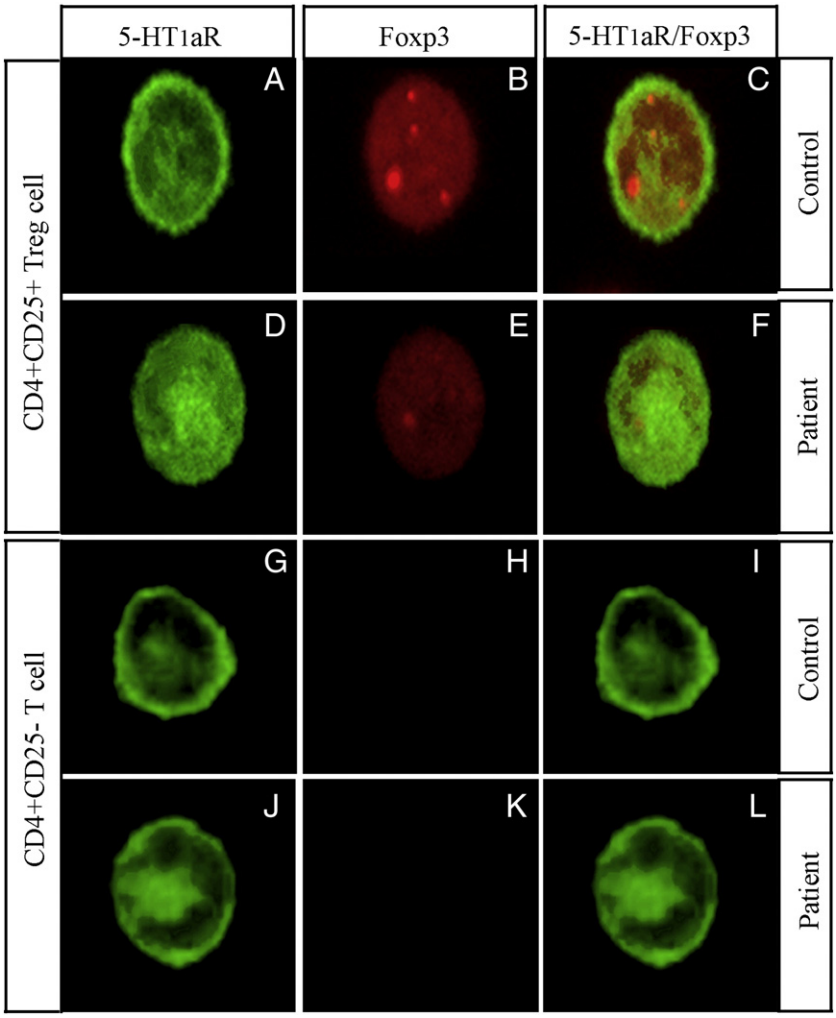

Fig. 4. Comparison of 5-HT_{1a}R and Foxp3 between major depression patients and healthy controls. (A–C) In $CD4^+CD25^+$ Treg cells of health controls, 5-HT_{1a}R was expressed most significantly on the membrane and less evident in the cytoplasm, and Foxp3 labeling was expressed throughout the nuclei and cytoplasm. (D–F) In $CD4^+CD25^+$ Treg cells of patients with major depression, both 5-HT_{1a}R and Foxp3 showed evident decrease. The decrease of 5-HT_{1a}R appeared to be most prominent on the membrane of $CD4^+CD25^+$ Treg cells. (G–I) In $CD4^+CD25^-$ T cells of healthy controls, 5-HT_{1a}R showed similar pattern as that in (A), while Foxp3 expression was not observed. (J–L) In $CD4^+CD25^-$ T cells of patients with major depression, the labeling of 5-HT_{1a}R and Foxp3 showed similar pattern as that in healthy controls (G–I).

$CD4^+CD25^+$ Treg cells. We found both the 5-HT and 5-HT_{1a}R mRNA showed significant correlation with $CD4^+CD25^+$ Treg cell counts ($r=0.836$, $r=0.793$, respectively; $p<0.01$), the percentage of $CD4^+CD25^+$ Treg cells in peripheral lymphocytes ($r=0.823$, $r=0.788$, respectively; $p<0.01$), and Foxp3 mRNA expression ($r=0.940$, $r=0.912$, respectively; $p<0.01$) (Table 3).

Table 3
Correlation analysis between $CD4^+CD25^+$ Treg cells and peripheral cytokines, 5-HT system.

	$CD4^+CD25^+$ Treg cells	$CD4^+CD25^+$ Treg cells/lymphocytes	Foxp3 mRNA
Peripheral cytokins			
IL-2	0.416**	0.446**	0.307*
IL-10	−0.125	−0.123	−0.316*
TGF-ß$_1$	0.517**	0.588**	0.449**
5-HT system			
5-HT	0.836**	0.823**	0.940**
5-HT_{1a}R mRNA	0.793**	0.788**	0.912**

* $p<0.05$.
** $p<0.01$.

4. Discussion

In the current study, we found an increased Th1/Th2 ratio and a decreased $CD4^+CD25^+$ Treg cell population in the peripheral blood lymphocytes of patients who were diagnosed with major depression for the first time. Furthermore, we observed a lower level of plasma 5-HT and 5-HT_{1a}R in peripheral blood lymphocytes in these patients compared to that of healthy controls, and interestingly, 5-HT_{1a}R expression showed a specific decrease in the $CD4^+CD25^+$ Treg cells. Our findings demonstrated that the immune dysfunction existed in the patients of major depression. In addition, the substantial decrease of 5-HT_{1a}R in $CD4^+CD25^+$ Treg cells

highlighted the possible interaction between the neurotransmitter 5-HT and $CD4^+CD25^+$ Treg cells and their involvement in immune imbalance of major depression.

Similar to previous studies (Maes et al., 1993, 1995; Kubera et al., 2001; Kim et al., 2002; Myint et al., 2005; Dimopoulos et al., 2008), our results indicated that immune imbalance existed in patients of major depression at cytokine levels, as early as their first onset. In order to observe the levels of Th1, Th2 and Th3 cells, we measured their major plasma cytokines expression of IL-2, IL10 and TGF-β_1. We found that the IL-2 level, a pro-inflammatory cytokine, was higher than that of normal healthy individuals, while the IL-10 and TGF-β_1 prominently decreased. Different from our findings, one other study, which recruited both first onset and recurrent major depression patients, showed that cytokine levels of both Th1 and Th2 cells were increased (Myint et al., 2005). A possibility for this difference could be the variety of subtypes of major depression. The aforementioned study did not distinguish between the major depression subtypes, while in our study the majority (around 80%) of the recruited patients belonged to the melancholic subtype; and different immune patterns have been found in melancholic major depression versus non-melancholic major depression, which was actually consistent with our results (Rothermundt et al., 2001). However, the tendency of the Th1/Th2 ratio shifting toward Th1 cells in the patients of our study was similar to previous studies (e.g. Myint et al., 2005), which indicated that an immune imbalance status existed in patients of major depression.

The immune imbalance in major depression patients was not only exhibited by the changes of cytokine levels and the suggested imbalance between Th1 and Th2 cells, but was also exhibited by decreased Treg cell population in peripheral blood lymphocytes, which could help with further understanding of the mechanism of immune dysregulation. In our study we focused on the $CD4^+CD25^+$ Treg cells, which are acknowledged to be the major population of Treg cells. We found that both the absolute number of peripheral $CD4^+CD25^+$ Treg cells and the percentages of $CD4^+CD25^+$ Treg cells in peripheral lymphocytes were decreased in the patients of major depression. This was interesting since the current findings were the first to demonstrate the change of $CD4^+CD25^+$ Treg cells in major depression patients, and we also found its significant correlation with the Th cytokines. $CD4^+CD25^+$ Treg cells could suppress the proliferation and/or cytokine production of $CD4^+$T cells (Annacker et al., 2001). It has also been shown that its deletion could result in the development of a rapidly lethal autoimmune syndrome both in rodents and humans (Sakaguchi et al., 1995), while its increase could benefit treating autoimmune diseases (Hauben et al., 2008). All these indicated the potential contribution of decreased $CD4^+CD25^+$ Treg cell level to the immune imbalance of major depression.

To further confirm the changes of $CD4^+CD25^+$ Treg cells in the major depression patients, we also determined the Foxp3 expression. The Foxp3 level showed consistent decrease in both the $CD4^+CD25^+$ Treg cells and the whole peripheral lymphocytes of the major depression patients, while its expression was not evident in $CD4^+CD25^-$ T cells. Foxp3 is an indispensable factor of $CD4^+CD25^+$ Treg cell development and function (Hori et al., 2003) in a dose-dependent rather than non-binary manner (Wan and Flavell, 2007). Continued Foxp3 expression in mature Treg cells is needed to maintain the transcriptional and functional program established during Treg cell development (Williams and Rudensky, 2007). Hence the alteration of Foxp3 expression further supported the potential functional detention of $CD4^+CD25^+$ Treg cells in patients of major depression.

We then explored the potential correlation of the 5-HT system and the Treg cells, in order to further understand the mechanisms of immune imbalance in major depression. The reason we focused on the 5-HT system was not only because the central role of 5-HT in major depression and suicide is well acknowledged (Middlemiss et al., 2002), but also because 5-HT acted as an immune modulator in the periphery (Mössner and Lesch, 1998): the reduced 5-HT level has been shown to diminish the activation potential of $CD4^+$ T cells, and impaired serotonergic transduction system was also indicated in immune cells of major depression (Mizrahi et al., 2004). 5-HT is involved in the pathogenesis of major depression through several receptors in the brain, such as 5-HT_{1a}R, 5-HT_{1b}R, 5-HT_3R etc. While it served as a immune regulator, particularly for the promotion of T cell proliferation, a major target for 5-HT action appeared to be the 5-HT_{1a}R (Aune et al., 1993). In our study, we found that the levels of both 5-HT in the plasma and 5-HT_{1a}R in lymphocytes in the patients of major depression showed a noticeable decrease, and these changes showed significant positive correlation with the levels of $CD4^+CD25^+$ Treg cells or Foxp3, which indicated that 5-HT may interact with $CD4^+CD25^+$ Treg cells.

To further understand the interaction of the 5-HT system and $CD4^+CD25^+$ Treg cells, we examined the expression of 5-HT_{1a}R on $CD4^+CD25^+$ Treg cells and $CD4^+CD25^-$ T cells by co-localizing the 5-HT_{1a}R and Foxp3. We observed that in the $CD4^+CD25^+$ Treg cells of major depression patients, the 5-HT_{1a}R labeling was more significant in the cytoplasm but much less evident on the membrane compared to that of healthy controls, while the change of its labeling was not significant in $CD4^+CD25^-$ T cells. This specific change of 5-HT_{1a}R labeling indicated the internalization of 5-HT_{1a}R protein in $CD4^+CD25^+$ Treg cells and further confirmed the potential interaction between the 5-HT system and $CD4^+CD25^+$ Treg in major depression. It was interesting, even though it was beyond the scope of the present study, to understand how 5-HT_{1a}R interacted with $CD4^+CD25^+$ Treg cells and the mechanisms that hamper the trafficking of 5-HT_{1a}R from the cytoplasm to the membrane in $CD4^+CD25^+$ Treg cells. Whether it involved the changes of secondary signaling pathways, such as nuclear factor-kappaB signaling (Abdouh et al., 2004), or if it was related to the dynamic changes of cytoskeletons (Ganguly et al., 2008) that could also regulate the 5-HT_{1a}R signaling, needs to be further explored to explain the reduction of 5-HT_{1a}R in $CD4^+CD25^+$ Treg cells.

In summary, an immune active status existed in patients who were first diagnosed with major depression. This was shown by a decreased number of $CD4^+CD25^+$ Treg cells and an imbalance of Th1/Th2 ratio shifting toward the Th1 phenotype. We also found that patients showed a decreased plasma 5-HT level and 5-HT_{1a}R reduction in $CD4^+CD25^+$ Treg cells. These observations suggested that the alteration of $CD4^+CD25^+$ Treg cells and their 5-HT_{1a}R was involved in the immune system dysregulation of major depression. Further studies are needed in order to explore the functional changes of 5-HT_{1a}R in major depression and the details of interaction between 5-HT_{1a}R and Treg cells.

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Conflict of interest

The authors declare no conflict of interests.

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