International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 11 (6): 596-603, 2015
ISSN 1811-7775

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ijp.2015.596.603

Clozapine Regulates Cytokines, T-cell Subsets and Immunoglobulins Serum Levels in MK-801-Evoked Schizophrenia Rat

[1,2]Huanzhong Liu, [2]Han Cai, [3]Zhenhua Ren, [1]Jian Zhong and [1]Jun Li
[1]College of Pharmacy, Anhui Medical University, Hefei, 230032, China
[2]Anhui Mental Health Center, Hefei, 230022, China
[3]Department of Anatomy, Anhui Medical University, Hefei, 230032, China

ARTICLE INFO
Article History:
Received: March 24, 2015
Accepted: June 25, 2015

Corresponding Author:
Jun Li
College of Pharmacy,
Anhui Medical University,
Hefei, 230032, China
Tel: +8655165161001
Fax: +8655165161001

ABSTRACT
An association between inflammatory abnormalities and schizophrenia has been found repeatedly. In the current study, schizophrenia was induced by MK-801 (0.35 mg kg^{-1}, i.p.) twice a day for 2 weeks in rat pups. We found serum levels of IL-2, IL-6 and immunoglobulins were significantly higher than the normal level but TNF-α levels were significantly suppressed after the treatment of MK-801 in neonatal rats. Clozapine or haloperidol treatment could reverse immunoglobulins and TNF-α levels. However, clozapine, not haloperidol, did not recover serum levels of IL-2 and IL-6. T-cell subsets in peripheral blood of model rats were significantly increased in adulthood. On the contrary, the rate of CD4 (+)/CD8a (+) T cells was reduced significantly. After the treatment of clozapine or haloperidol, the levels of T-cell subsets were restored. However, clozapine, not haloperidol, has no effect on the rate of CD4 (+)/CD8a (+) T cells. These results suggested that the dysfunction of cytokine system and immune disturbance may be involved in the pathogenesis of MK-801-induced rat model of schizophrenia. The effects of clozapine and haloperidol on cytokines production, T-cell subsets switch and immunoglobulins presented in this study indicated that immunomodulatory therapy may be a potential strategy in the treatment of schizophrenia.

Key words: MK-801, Schizophrenia, T-cell subsets, immunoglobulins, cytokines

INTRODUCTION

The etiological explanations of schizophrenia were focused on disturbances of the dopaminergic neurotransmission. Unfortunately, the researches on dopamine system dysfunction did not achieve convincing results and antipsychotic antidopaminergic drugs still show unsatisfactory therapeutic effects (Horvath and Mirnics, 2014). New concepts in the pathophysiology research of schizophrenia are required. Recently, epidemiological, genetic, transcriptome, postmortem, peripheral biomarker and therapeutic studies of schizophrenia all pointed towards immune system abnormalities in patients suffering from schizophrenia and it is likely that these immune changes actively contribute to disease symptoms (Muller *et al*., 1999).

The abnormalities of immune system in schizophrenia, including dysregulation of cytokines production, T-cell subsets and immunoglobulins, have been one of the more enduring findings in the field over the last few decades, albeit with significant heterogeneity in the results, including negative studies (Rao *et al*., 1985; Villemain *et al*., 1989; Maes *et al*., 2000; Erbagci *et al*., 2001). Cytokines act as key players in the coordinate responses of cells in the innate immune system and the adaptive immune system (Potvin *et al*., 2008). The most frequently studied cytokines in schizophrenia are serum interleukin (IL)-1 β, IL-2, IL-6 and Tumor Necrosis Factor (TNF)-β (Shintani *et al*., 1991; Zhang *et al*., 2004; Soczynska *et al*., 2009; Gray and Bloch, 2012; Munkholm *et al*., 2013). Beside the results on IL-6 levels is with more consistency (Rao *et al*., 1985; Akiyama, 1999; Arolt *et al*., 2000), level of serum interleukin (IL)-1 β, IL-2 and TNF-α mentioned by many authors are still controversial (Cazzullo *et al*., 1998; Sperner-Unterweger *et al*., 1999; Ebrinc *et al*., 2002; Miller and Buckley, 2012).There are

also alterations in T-lymphocyte subsets in the peripheral blood of schizophrenic patients in acute psychosis, abnormal lymphocyte proliferative responses to mitogens and soluble antigens *in vitro* and changes in the numbers and percentages of T-cells, as well as T-cell subsets (Theodoropoulou *et al*., 2001; Haberny *et al*., 2002; Kocahan *et al*., 2013). Additionally, the alterations of IgG, IgA and IgM antibodies are still detected and most studies revealed that immunoglobulins levels had a change in schizophrenia rats and patients (Delisi *et al*., 1981; Reichelt and Landmark, 1995; Steiner *et al*., 2013), even though other studies showed that there was no significant difference of IgG levels between schizophrenia patients and controls (Emelia *et al*., 2012; Masdeu *et al*., 2012).

Clozapine, a dibenzodiazepine developed in 1961, is a unique antipsychotic, specifically indicated in the management of treatment resistant schizophrenia (Muller and Schwarz, 2010). However, it is not considered as a rest-line drug due to several potentially life threatening side-effects, including agranulocytosis, aspiration pneumonia, ileus, type II diabetes, myocarditis and cardiomyopathy (Viberg *et al*., 2008; Cunha *et al*., 2015). These effects seem to be associated with an immune response and the dysregulation of inflammatory cytokines such as IL-2, IL-6 and TNF-α (Sandberg *et al*., 2015).

Blockade of NMDA receptor during early neonatal life can cause long-term alterations in the anatomical, neurochemical, neurophysiological and behavioral properties of rodents (Haberny *et al*., 2002; Viberg *et al*., 2008; Kocahan *et al*., 2013). Therefore, blockade of the NMDA receptor with MK-801 during the early postnatal period has been proposed to be an experimental model of schizophrenia. However, there is unclear on the immunological alterations of animal models of schizophrenia induced by MK-801 during the early postnatal period and the immunological regulatory effects of clozapine are still understood. The aim of the current study was to assess the immunologic alteration in MK-801-evoked schizophrenia rats and to detect the effects of clozapine treatment on immunological parameters.

MATERIALS AND METHODS

Ethics statement: The experiments were performed at the Department of Anatomy of Anhui Medical university. The protocol was approved by the local ethics committees of Anhui Medical University. All efforts were made to minimize the number of animals used and to ameliorate any distress.

Housing: All rats were housed in ambient temperature (approx. 20-23°C) and humidity (approx. 60%) controlled vivarium. Food and water were available *ad libitum*, except during testing, when no food was provided. In all experiments, animals were allowed to habituate to the housing conditions for at least 1 week before behavioral testing. All testing was performed during the light phase of the day/night cycle.

Animal administration: Sprague-Dawley pups that were fostered by their real mothers (n = 8) and there were 5-6 offspring per litter. The pups were randomly divided into four groups, MK-801 (M group), MK-801+Clozapine (CL group), MK-801+Haloperidol (H group) and normal control group (N group) and each group included eleven Sprague-Dawley pups. All pups from a given litter received the same treatment and were randomly assigned to the groups, so that mean body weight in each group was almost equal on PND 7. The pups of M group, CL group and H group received i.p. injections of the NMDA receptor antagonist dizocilpine (MK-801; 0.35 mg kg^{-1}; SIGMA) and N group received i.p. injections of vehicle (0.9% NaCl) twice a day within 8:00-10:00 am and 14:00- 16:00 pm of the light Phase from Postnatal Days (PND) 6-21 of age. The animals were weaned at day 21. The animals of CL group and H group were treated with the administration of clozapine (1 mg kg^{-1} SIGMA), or Haloperidol (0.1 mg kg^{-1}, Hunan Dongting Pharm. Co. Ltd.) and N group and M group received an equal volume of vehicle (0.9% NaCl) once a day within 14:00-15:00 pm on PND 43 and PND 57 for two weeks.

Flow cytometry: Animals were anesthetized by 10% hydral on PND 90. After anesthesia, the common carotid arteries were isolated from the rats and 4 mL blood sample were collected from rat common carotid artery in tube anticoagulated with heparin. According to the protocol of Ficoll-Paque TM PLUS (Catalog No. 07957, StemcCell Technologies), the mononuclear cells were isolated from peripheral blood. The blood were Diluted with Phosphate Buffered Saline plus (DPBS) (volume ratio 1:1) and then carefully layered onto the medium to minimize mixing of blood with ficoll. The cells were harvested by carefully pipetting them from the liquid interface after the centrifugalization at room temperature (15-25°C) at 2000 rmp for 20 min and then washed in DPBS and centrifuged at 1500 rmp for 20 min. The supernatant was then abandoned and the cells were resuspended in 300 μL DPBS. Flow cytometry (FACScalibur, BD Biosciences) was performed with the following antibodies, FITC anti-rat CD3 0.5 μL (201403, Biolegend), APC anti-rat CD4 1.25 μL (201509, Biolegend) and PE anti-rat CD8a 1.25 μL (200607, BioLegend). Data was analyzed with WinMDI 2.9 software.

ELISA analysis: Two milliliter blood samples were collected and serum was got after centrifugalization at 4 , 4000 rmp for 20 min and stored at -80°C. To assess plasma levels of IL-6, IL-1 β, IL-2, TNF-α, IgA, IgM and IgG, Enzyme-Linked Immunosorbent Assay (ELISA) kits are used according to the manufacturer's instructions. The details of ELISA kits was shown in Table 1.

Table 1: Details of ELISA kits

ELISA kits	Lot No.	Manufacturers
Rat IL-6	ELR-IL6-001	Raybiotech
Rat IL-1 beta	ELR-IL1beta-001	Raybiotech
Rat IL-2	ELR-IL2-001	Raybiotech
Rat TNF-alpha	ELR-TNFalpha-001	Raybiotech
Rat IgA	88-50480-22	eBioscience
Rat IgM	88-50540-22	eBioscience
Rat IgG	88-50490-22	eBioscience

IL: Interleukin, TNF: Tumor necrosis factor, ELISA: Enzyme linked immunosorbent assay

Statistical analysis: All data analysis was conducted using SPSS 16.0 for Windows. The values was presented as the Means±SD. All data of water maze was analyzed with repeated measures ANOVA, followed by Tukey-kramer HSD post hoc test to compare means of interest. The two study groups were compared for continuous variables by an independent t test. The pre- and post- treatment data were compared by the paired t test. Two-tailed $p<0.05$ was considered significant.

RESULTS

Serum levels of cytokines in schizophrenia rats: As shown in Fig. 1, after the treatment of MK-801 in neonatal rats, serum levels of IL-2 (Fig. 1d) and IL-6 (Fig. 1c) were significantly increased compared with normal level but TNF-α levels were significantly down regulated in M group ($p<0.01$) (Fig. 1b). Of note, IL-1β serum levels had no significant difference between N group and M group ($p>0.05$) (Fig. 1a).

Effects of clozapine and haloperidol on cytokines in schizophrenia rats: Interestingly, after treated with clozapine, serum concentrations of IL-2 and IL-6 were increased significantly compared to M group ($p<0.01$) (Fig. 1c-d). However, the levels of TNF-α were only increased to normal level ($p<0.05$) (Fig. 1b). Similarly, IL-2, IL-6 and TNF-alpha concentrations were all recovered to normal level in haloperidol group compared to N group ($p<0.01$) (Fig. 1b-d). Serum level of IL-1β had no changes after the treatment of clozapine or haloperidol (Fig. 1a).

T-cell subsets of peripheral blood in schizophrenia rats: T-cell subsets in peripheral blood of model rats were detected by flow cytometer and results showed that, compared with N group, CD3 (+), CD8a (+), CD4 (+) T cell levels in M group were significantly increased ($p<0.01$) (Fig. 2a-c). Similarly, the levels of CD3 (+) CD8a (+) and CD3 (+) CD4 (+) double-positive T cells were also high expressed in M group compared with N group ($p<0.01$) (Fig. 2d-e). In contrast, the rate of CD4 (+)/CD8a (+) T cells was reduced significantly in M group compared with N group ($p<0.01$) (Fig. 2f).

Effects of clozapine and haloperidol on T-cell subsets in schizophrenia rats: In comparison with M group, significant low-expression of CD3 (+), CD4 (+), CD8a (+), CD3 (+) CD8a (+) and CD3 (+) CD4 (+) T-cell subsets were detected in peripheral blood after clozapine treatment ($p<0.01$) (Fig. 2a-e). Moreover, compared with N group, the levels of CD3 (+), CD3 (+) CD8a (+) and CD3 (+) CD4 (+) T-cell subsets of CL group were recovered ($p>0.05$) but CD4 (+) and CD8 (+) in CL group were still significantly higher than that

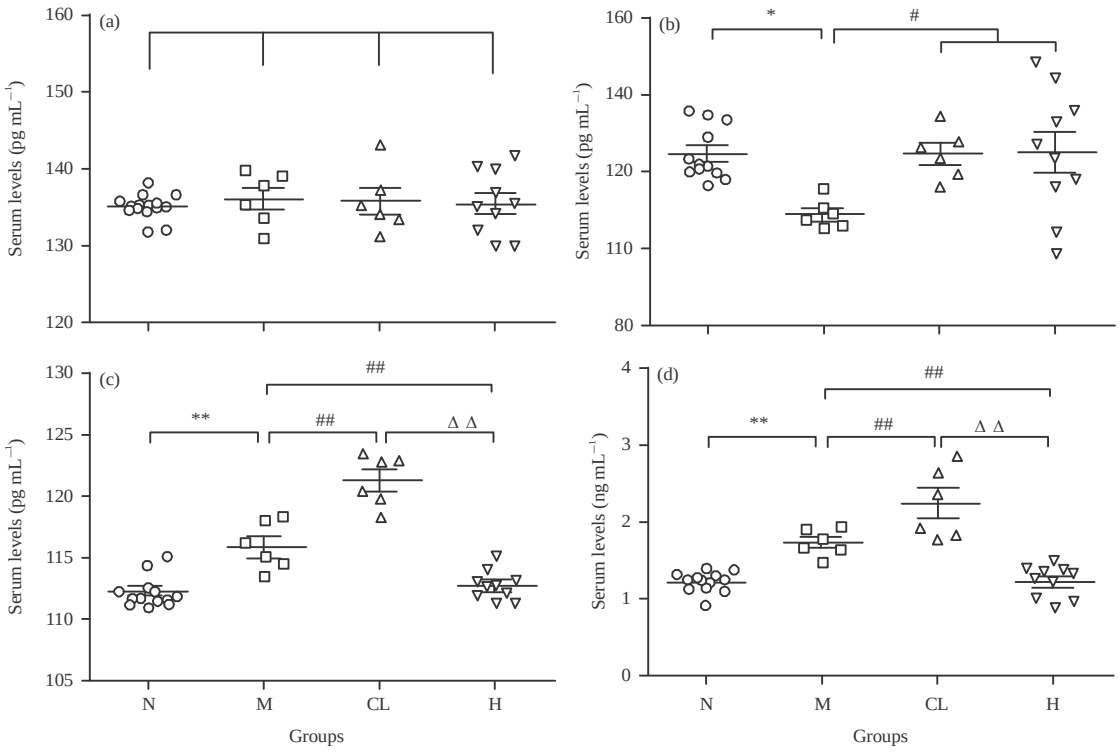

Fig. 1(a-d): Effects of clozapine and haloperidol on serum cytokines in schizophrenia rats (n = 11), N: Normal group, M: Model group, CL: Clozapine group, H: Haloperidol group. Compared with N group, **$p<0.01$, *$p<0.05$, Compared with M group, $^{\#\#}p<0.01$, $^{\#}p<0.05$, Comparison between CL groups and H group, $^{\Delta\Delta}p<0.01$, $^{\Delta}p<0.05$, (a) IL-1β, (b) TNF-α, (c) IL-6 and (d) IL-2

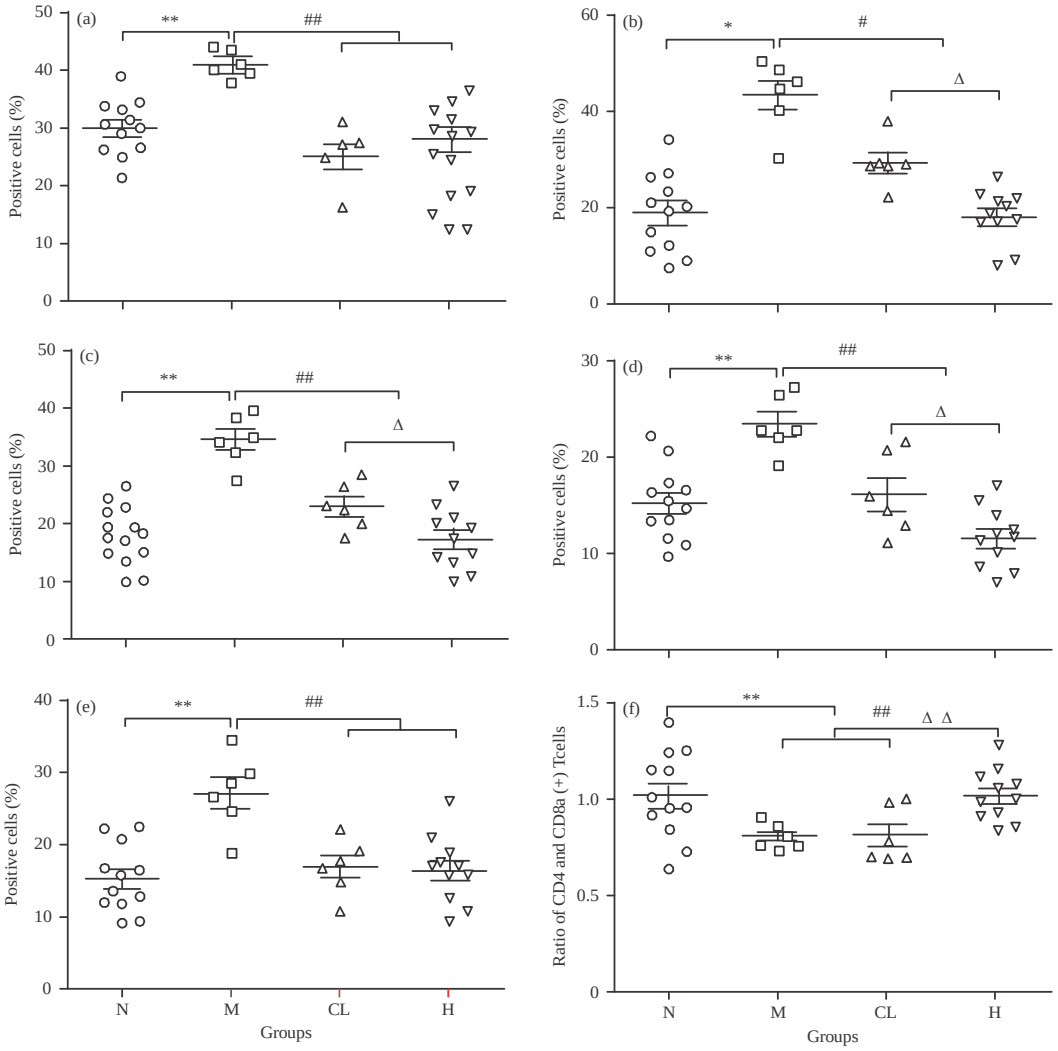

Fig. 2(a-f): Effects of clozapine and haloperidol on T-cell subsets in schizophrenia rats (n = 11), (a) and (c) T-cell subsets in peripheral blood, (b) and (d) CD4 (+)/CD8a (+) T cells in peripheral blood. N: Normal group, M: Model group, CL: Clozapine group, H: Haloperidol group, compared with N group, **p<0.01, *p<0.05, Compared with M group, $^{\#\#}$p<0.01, $^{\#}$p<0.05, Comparison between CL groups and H group, $^{\Delta\Delta}$p<0.01, $^{\Delta}$p<0.05

in N group (p<0.05) (Fig. 2a-e). However, the rate of CD4 (+)/CD8a (+) T cells in peripheral blood of CL group was still lower than N group (p<0.01) (Fig. 2f).

Similarly, haloperidol treatment was also able to reduce the numbers of CD3 (+), CD4 (+), CD8a (+), CD3 (+) CD8a (+) and CD3 (+) CD4 (+) T-cell subsets in peripheral blood (p<0.01) (Fig. 2a-e). However, compared with CL group, the levels of CD4 (+) and CD8 (+) T cells in H group were decreased substantially (p<0.05) (Fig. 2b-c). The levels of T-cell subsets were all recovered to normal compared with N group (p>0.05) (Fig. 2a-e). The rate of CD4 (+)/CD8a (+) T cells was investigated as well in haloperidol group. Although the ratio in H group was still lower than normal levels, it was significantly higher than that in M and CL group (p<0.01) (Fig. 2f). The results suggested that, treatment with haloperidol, unlike clozapine, had partially restored the rate of CD4 (+)/CD8a (+) T cells in schizophrenia rats.

Serum levels of immunoglobulins in schizophrenia rats: Serum levels of immunoglobulins were measured by ELISA and results showed, compared to N group, concentrations of IgA, IgM and IgG were significantly up regulated in M group (p<0.01) (Fig. 3a-c).

Effects of clozapine and haloperidol on immunoglobulin in schizophrenia rats: In comparison with M group, serum concentrations of IgA, IgM and IgG were markedly decreased after clozapine and haloperidol treatment (p<0.01) (Fig. 3a-c). IgA IgM and IgG serum concentrations in H group had no significant difference compared to N group (p>0.05) (Fig. 3a-c). However, after the treatment of clozapine, IgG and IgM serum concentrations in CL group decreased more significantly than that in H group (p<0.05) (Fig. 3b-c).

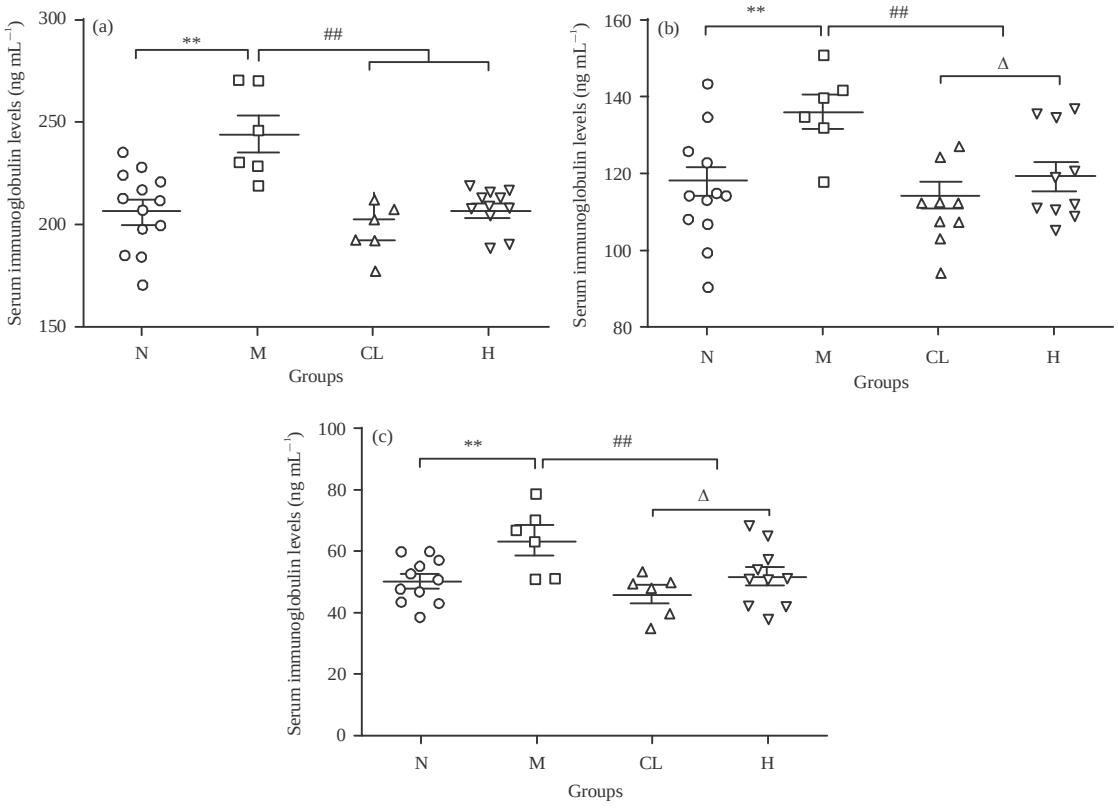

Fig. 3(a-c): Effects of clozapine and haloperidol on Immunoglobulin in schizophrenia rats (n = 11), N: Normal group, M: Model group, CL: Clozapine group, H: Haloperidol group. Compared with N group, **p<0.01, *p<0.05, compared with M group, $^{\#\#}$p<0.01, $^{\#}$p<0.05, Comparison between CL groups and H group, $^{\Delta\Delta}$p<0.01, $^{\Delta}$p<0.05

DISCUSSION

Schizophrenia (SZ) is a chronic and severe CNS disease affecting ~1% of the population (Perala *et al*., 2007). Recently, there is a growing body of evidence that points to dysfunction of the immune system in patients with SZ (Pathmanandavel *et al*., 2013). Up to now, although clozapine is also the most effective drug in schizophrenia treatment on clinic, its use is limited because of the high incidence of adverse reactions (Jules *et al*., 2012). However, the pathophysiology of SZ and the mechanism of clozapine-induced adverse reactions remain mysterious. In this study, we aimed to explore the immunologic alteration in MK-801-evoked schizophrenia rats during the clozapine-treated process.

Cytokines: Cytokines are pleotrophic proteins that coordinate the host response to infection as well as mediate normal, ongoing signaling between cells of non-immune tissues, including the nervous system (Deverman and Patterson, 2009). The production and release of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α are typically intended to prevent further damage to CNS tissue, they may also be toxic to neurons and other glial cells, which might be involved in the pathogenesis and pathophysiology of schizophrenia (Boulanger and Shatz, 2004; Drexhage *et al*., 2011). In particular, interleukins are involved in a variety of neural processes and have a relationship with psychiatric disorders (Malek-Ahmadi, 1996; Boulanger and Shatz, 2004; Nawa and Takei, 2006). However, series of studies found that abnormal expression of interleukins in the plasma, brain tissues, or cerebrospinal fluid of SZ patients (Theodoropoulou *et al*., 2001; Potvin *et al*., 2008; Soderlund *et al*., 2009). However, there has been significant heterogeneity for cytokines expression among studies (Watanabe *et al*., 2010). Our results showed serum levels of IL-1β were not affected in schizophrenia rats with or without clozapine treatment, which was consistent with most of studies (Baker *et al*., 1996; Toyooka *et al*., 2003; Potvin *et al*., 2008), although Song *et al*. (2009) found that IL-1β serum levels and mRNA expression in peripheral blood mononuclear cell were increased in rest-episode schizophrenia patients.

Moreover, we also found that the serum level of TNF-α was decreased in schizophrenia rats and recovered to normal after treatment of clozapine and haloperidol. Similarly, Haack drew a same conclusion that TNF-α concentrations were lower in schizophrenia patients than controls (Haack *et al*., 1999). On the contrary, elevated serum or plasma levels of TNF-α are one of the most frequently confirmed immunological features associated with schizophrenia (Akiyama, 1999; Garcia-Miss *et al*., 2010; Kubistova *et al*., 2012; Song *et al*., 2013). Alzbeta (Kubistova *et al*., 2012) also reported that the

plasma level of TNF-α did not show any decrease after antipsychotics treatment in patients with drug naive, rest episode schizophrenia while Miller *et al*. (2011) found TNF-α levels remained elevated in acute exacerbations and following antipsychotics treatment. Interestingly, although results from many investigations indicated that serum IL-6 level is elevated or decreased after antipsychotics treatment in patients (Akiyama, 1999; Garcia-Miss *et al*., 2010; Kubistova *et al*., 2012; Song *et al*., 2013), our results detected a higher expression of TNF-α in model group of rats while treatment of haloperidol decreased but treatment of clozapine significantly increased its expression.

The IL-2 is a T-cell growth factor and has been shown to modulate a range of psychiatric manifestations. Some reports (Ganguli *et al*., 1995; Singh *et al*., 2011; Liu *et al*., 2012) showed that a lower levels of IL-2 were observed among the schizophrenia patients but others (Ebrinc *et al*., 2002; Zhang *et al*., 2002; Muller and Schwarz, 2006; Potvin *et al*., 2008; Zhang *et al*., 2009) found that increased IL-2 levels were observed in the serum, plasma and cerebrospinal fluid of schizophrenic patients. Moreover, another study (Kim *et al*., 2000) has also found close relationships between IL-2 and schizophrenic symptomatology. In our study, we found that elevated serum level of IL-2 in M group and it was recovered to normal after haloperidol treatment but increased significantly after clozapine treatment.

T-cell subsets; T Cell-mediated immunity is an adaptive process of developing antigen-specific T lymphocytes to eliminate viral, bacterial, or parasitic infections or malignant cells. Its functions depend on the total T lymphocytes (CD3+) and its subsets (CD4+, CD8+) relative composition (Broere *et al*., 2011). Treg subsets are presented within both the CD4+ and the CD8+ T cell compartments. It has been characterized that the CD8+ Treg alterations in autoimmune diseases (Filaci *et al*., 2011). The enumeration of CD4 and CD8 positive cells, surrogate markers for HIV disease progression, is helpful in management and follow up of immunocompromised patients (Ray *et al*., 2006). Nyland *et al*. (1980) reported that both immune mechanisms and the neuroleptic drug treatment may be of importance for the observed decrease in T lymphocyte numbers in blood from patients with acute schizophrenia. The animal studies showed that peripheral T cell deficit could lead to cognitive and behavioral impairment, highlighting the importance of properly functioning adaptive immunity in the maintenance of mental activity and in coping with conditions leading to cognitive deficits (Kipnis *et al*., 2004). Our current study detected a significant increase of CD3+CD8a+CD4+ CD3+CD8a+ and CD3+CD4+ T cells but a lower ratio of CD4+/CD8a+ T cell in peripheral blood of model rats. However, the T cell subsets were all recovered except for the ratio of CD4+/CD8a+ T cells after clozapine and haloperidol treatment, indicating that clozapine treatment were able to attenuate the disturbances in T cell subgroup immunity that was occurred in schizophrenia rats.

Immunoglobulin: The present findings of elevated IgG, IgA and IgM antibodies in schizophrenia rats are in agreement with Steiner *et al*. (2013). Similarly, the previous studies also found a higher levels of IgA in patients with schizophrenia above the upper normal limits (Reichelt and Landmark, 1995). On the contrary, Delisi *et al*. (1981) detected a generalized reduction of lgG, IgA and IgM serum levels in the schizophrenic patients, which was not consistent with our data. However, other studies showed that there was no significant difference of IgG levels between schizophrenia patients and controls (Emelia *et al*., 2012; Masdeu *et al*., 2012). In our study, we also found that levels of IgG, IgA and IgM were markedly decreased after clozapine and haloperidol treatment.

The main finding of this study was demonstration that neonatal exposure to MK-801 led to long-lasting immunological disturbances in adult, evident already in 28 days old male rats. This clearly indicates that immunological changes in this animal model precede the behavioral deficit and suggests functional engagement of the immune system in the development of schizophrenia-like behavioral symptoms. Furthermore, Clozapine treatment was able to reverse some immunological parameters, has no significant impact on other indexes. These might be related to its therapeutic effects and adverse reactions.

In summary, this study demonstrated that peripheral immunological changes in the MK-801-evoked model of schizophrenia were long-lasting and attenuated by clozapine treatment. Moreover, the disturbances in T cell-mediated immunity as well as cytokine production. Thus, our data support the hypothesis that prenatal immune over activation can be a causative factor in pathogenesis of schizophrenia and effects of clozapine may be associated with immunological regulation.

ACKNOWLEDGMENTS

We thank Ph.D Xu Hui for the assistance of behavioral detection; Profession Li Guangwu for valuable inspiration and comments. This research was supported by the Science Foundation of Hefei (20112562) and the Natural Science Foundation of China (81372693 81273526).

REFERENCES

Akiyama, K., 1999. Serum levels of soluble IL-2 receptor α, IL-6 and IL-1 receptor antagonist in schizophrenia before and during neuroleptic administration. Schizophrenia Res., 37: 97-106.

Arolt, V., M. Rothermundt, K.P. Wandinger and H. Kirchner, 2000. Decreased *in vitro* production of interferon-gamma and interleukin-2 in whole blood of patients with schizophrenia during treatment. Mol. Psychiatry, 5: 150-158.

Baker, I., J. Masserano and R.J. Wyatt, 1996. Serum cytokine concentrations in patients with schizophrenia. Schizophr. Res., 20: 199-203.

Boulanger, L.M. and C.J. Shatz, 2004. Immune signalling in neural development, synaptic plasticity and disease. Nat. Rev. Neurosci., 5: 521-531.

Broere, F., S.G. Apasov, M.V. Sitkovsky and W. van Eden, 2011. A2 T Cell Subsets and T Cell-mediated Immunity. In: Principles of Immunopharmacology, Nijkamp, F.P. and M.J. Parnham (Eds.). Birkhauser Basel, New York, pp: 15-27.

Cazzullo, C.L., M. Saresella, K. Roda, M.G. Calvo and P. Bertrando *et al*., 1998. Increased levels of CD8+ and CD4+ 45RA+ lymphocytes in schizophrenic patients. Schizophr. Res., 31: 49-55.

Cunha, B.M., L.M.H. Mota, G.S. Pileggi, I.P. Safe and M.V.G. Lacerda, 2015. HIV/AIDS and rheumatoid arthritis. Autoimmun Rev., 14: 396-400.

Delisi, L.E., D.R. Weinberger, S. Potkin, L.M. Neckers, D.J. Shiling and R.J. Wyatt, 1981. Quantitative determination of immunoglobulins in CSF and plasma of chronic schizophrenic patients. Br. J. Psychiatry, 139: 513-518.

Deverman, B.E. and P.H. Patterson, 2009. Cytokines and CNS development. Neuron, 64: 61-78.

Drexhage, R.C., K. Weigelt, N. van Beveren, D. Cohen, M.A. Versnel, W.A. Nolen and H.A. Drexhage, 2011. Immune and neuroimmune alterations in mood disorders and schizophrenia. Int. Rev. Neurobiol., 101: 169-201.

Ebrinc, S., C. Top, O. Oncul, C. Basoglu, S. Cavuslu and M. Cetin, 2002. Serum interleukin 1 alpha and interleukin 2 levels in patients with schizophrenia. J. Int. Med. Res., 30: 314-317.

Emelia, O., R.N. Amal, Z.Z. Ruzanna, H. Shahida and Z. Azzubair *et al*., 2012. Seroprevalence of Anti-toxoplasma gondii IgG antibody in patients with schizophrenia. Trop. Biomed., 29: 151-159.

Erbagci, A.B., H. Herken, O. Koyluoglu, N. Yilmaz and M. Tarakcioglu, 2001. Serum IL-1beta, sIL-2R, IL-6, IL-8 and TNF-alpha in schizophrenic patients, relation with symptomatology and responsiveness to risperidone treatment. Mediators Inflamm., 10: 109-115.

Filaci, G., D. Fenoglio and F. Indiveri, 2011. $CD8^+$ T regulatory/suppressor cells and their relationships with autoreactivity and autoimmunity. Autoimmunity, 44: 51-57.

Ganguli, R., J.S. Brar, K.R.N. Chengappa, M. DeLeo and Z.W. Yang *et al*., 1995. Mitogen-stimulated interleukin-2 production in never-medicated, First-episode schizophrenic patients: The influence of age at onset and negative symptoms. Arch. Gen. Psychiatry, 52: 668-672.

Garcia-Miss, M.R., J. Perez-Mutul, B. Lopez-Canul, F. Solis-Rodriguez and L. Puga-Machado *et al*., 2010. Folate, homocysteine, interleukin-6 and tumor necrosis factor alfa levels, but not the methylenetetrahydrofolate reductase C677T polymorphism, are risk factors for schizophrenia. J. Psychiatr Res., 44: 441-446.

Gray, S.M. and M.H. Bloch, 2012. Systematic review of proinflammatory cytokines in obsessive-compulsive disorder. Curr. Psychiat. Rep., 14: 220-228.

Haack, M., D. Hinze-Selch, T. Fenzel, T. Kraus, M. Kuhn, A. Schuld and T. Pollmacher, 1999. Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: Effects of confounding factors and diagnosis. J. Psychiatr. Res., 33: 407-418.

Haberny, K.A., M.G. Paule, A.C. Scallet, F.D. Sistare, D.S. Lester, J.P. Hanig and W. Slikker Jr., 2002. Ontogeny of the N-methyl-D-aspartate (NMDA) receptor system and susceptibility to neurotoxicity. Toxicol. Sci., 68: 9-17.

Horvath, S. and K. Mirnics, 2014. Immune system disturbances in schizophrenia. Biol. Psychiat., 75: 316-323.

Jules, J., P. Zhang, J.W. Ashley, S. Wei and Z. Shi *et al*., 2012. Molecular basis of requirement of receptor activator of nuclear factor κB signaling for interleukin 1-mediated osteoclastogenesis. J. Biol. Chem., 287: 15728-15738.

Kim, Y.K., L. Kim and M.S. Lee, 2000. Relationships between interleukins, neurotransmitters and psychopathology in drug-free male schizophrenics. Schizophr. Res., 44: 165-175.

Kipnis, J., H. Cohen, M. Cardon, Y. Ziv and M. Schwartz, 2004. T cell deficiency leads to cognitive dysfunction: Implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. Proc. Natl. Acad. Sci. USA., 101: 8180-8185.

Kocahan, S., K. Akillioglu, S. Binokay, L. Sencar and S. Polat, 2013. The effects of N-Methyl-D-Aspartate receptor blockade during the early neurodevelopmental period on emotional behaviors and cognitive functions of adolescent Wistar rats. Neurochem. Res., 38: 989-996.

Kubistova, A., J. Horacek and T. Novak, 2012. Increased interleukin-6 and tumor necrosis factor alpha in first episode schizophrenia patients versus healthy controls. Psychiat. Danub., 24: S153-S156.

Liu, H., Y. Kang, J. Liang, C. Li and M. Xiu *et al*., 2012. Lower serum interleukin-2 levels in schizophrenic patients with tardive dyskinesia. Psychiat. Res., 198: 329-331.

Maes, M., L.B. Chiavetto, S. Bignotti, G.J.B. Tura and R. Pioli *et al*., 2000. Effects of atypical antipsychotics on the inflammatory response system in schizophrenic patients resistant to treatment with typical neuroleptics. Eur. Neuropsychopharmacol., 10: 119-124.

Malek-Ahmadi, P., 1996. Neuropsychiatric aspects of cytokines research: An overview. Neurosci. Biobehav. Rev., 20: 359-365.

Masdeu, J.C., A. Gonzalez-Pinto, C. Matute, S.R. de Azua, A. Palomino *et al*., 2012. Serum IgG antibodies against the NR1 subunit of the NMDA receptor not detected in schizophrenia. Am. J. Psychiatry, 169: 1120-1121.

Miller, B.J., P. Buckley, W. Seabolt, A. Mellor and B. Kirkpatrick, 2011. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol. Psychiat., 70: 663-671.

Miller, B.J. and P.F. Buckley, 2012. Is relapse in schizophrenia an immune-mediated effect? FOCUS J. Lifelong Learn. Psychiatry, 10: 115-123.

Muller, N., M. Riedel, M. Ackenheil and M.J. Schwarz, 1999. The role of immune function in schizophrenia: An overview. Eur. Arch. Psychiatry Clin. Neurosci., 249: 62-68.

Muller, N. and M. Schwarz, 2006. Schizophrenia as an inflammation-mediated dysbalance of glutamatergic neurotransmission. Neurotox. Res., 10: 131-148.

Muller, N. and M.J. Schwarz, 2010. Immune system and schizophrenia. Curr. Immunol. Rev., 6: 213-220.

Munkholm, K., M. Vinberg and L.V. Kessing, 2013. Cytokines in bipolar disorder: A systematic review and Meta-analysis. J. Affect. Disord, 144: 16-27.

Nawa, H. and N. Takei, 2006. Recent progress in animal modeling of immune inflammatory processes in schizophrenia: Implication of specific cytokines. Neurosci. Res., 56: 2-13.

Nyland, H., A. Naess and H. Lunde, 1980. Lymphocyte subpopulations in peripheral blood from schizophrenic patients. Acta Psychiat. Scand., 61: 313-318.

Pathmanandavel, K., J. Starling, R.C. Dale and F. Brilot, 2013. Autoantibodies and the immune hypothesis in psychotic brain diseases: Challenges and perspectives. Clin. Dev. Immunol., 10.1155/2013/257184

Perala, J., J. Suvisaari, S.I. Saarni, K. Kuoppasalmi and E. Isometsa *et al*., 2007. Lifetime prevalence of psychotic and bipolar I disorders in a general population. Arch. Gen. Psychiatry, 64: 19-28.

Potvin, S., E. Stip, A.A. Sepehry, A. Gendron, R. Bah and E. Kouassi, 2008. Inflammatory cytokine alterations in schizophrenia: A systematic quantitative review. Biol. Psychiatry, 63: 801-808.

Rao, I.V.L.N., P.S. Gopinath, N. Jayasimha, B.S.S.R. Rao and D.K. Subbakrishna, 1985. Serum immunoglobulins and schizophrenia. Indian J. Psychiat., 27: 325-328.

Ray, K., S.M. Gupta, M. Bala, S. Muralidhar and J. Kumar, 2006. CD4/CD8 lymphocyte counts in healthy, HIV-positive individuals and AIDS patients. Indian J. Med. Res., 124: 319-330.

Reichelt, K.L. and J. Landmark, 1995. Specific IgA antibody increases in schizophrenia. Biol. Psychiat., 37: 410-413.

Sandberg, M.E.C., C. Bengtsson, L. Klareskog, L. Alfredsson and S. Saevarsdottir, 2015. Recent infections are associated with decreased risk of rheumatoid arthritis: A population-based case-control study. Ann. Rheum. Dis., 10.1136/annrheumdis-2014-206493

Shintani, F., S. Kanba, N. Maruo, T. Nakaki and M. Nibuya *et al*., 1991. Serum interleukin-6 in schizophrenic patients. Life Sci., 49: 661-664.

Singh, B., N.K. Bera, C.R. Nayak and T.K. Chaudhuri, 2011. Immunomodulation in schizophrenia: A study among the Indian schizophrenia patients of Siliguri, West Bengal. Asian J. Psychiatry, 4: 277-283.

Soczynska, J.K., S.H. Kennedy, B.I. Goldstein, A. Lachowski, H.O. Woldeyohannes and R.S. McIntyre, 2009. The effect of tumor necrosis factor antagonists on mood and mental health-associated quality of life: Novel hypothesis-driven treatments for bipolar depression? Neurotoxicology, 30: 497-521.

Soderlund, J., J. Schroder, C. Nordin, M. Samuelsson and L. Walther-Jallow *et al*., 2009. Activation of brain interleukin-1β in schizophrenia. Mol. Psychiat., 14: 1069-1071.

Song, X., X. Fan, X. Song, J. Zhang and W. Zhang *et al*., 2013. Elevated levels of adiponectin and other cytokines in drug naive, first episode schizophrenia patients with normal weight. Schizophr. Res., 150: 269-273.

Song, X.Q., L.X. Lv, W.Q. Li, Y.H. Hao and J.P. Zhao, 2009. The interaction of nuclear factor-kappa B and cytokines is associated with schizophrenia. Biol. Psychiat., 65: 481-488.

Sperner-Unterweger, B., A. Whitworth, G. Kemmler, W. Hilbe, J. Thaler, G. Weiss and W.W. Fleischhacker, 1999. T-cell subsets in schizophrenia: A comparison between Drug-naive first episode patients and chronic schizophrenic patients. Schizophr. Res., 38: 61-70.

Steiner, J., M. Walter, W. Glanz, Z. Sarnyai and H.G. Bernstein *et al*., 2013. Increased prevalence of diverse *N*-methyl-D-aspartate glutamate receptor antibodies in patients with an initial diagnosis of schizophrenia specific relevance of IgG NR1a antibodies for distinction from *N*-methyl-D-aspartate glutamate receptor encephalitis. JAMA Psychiat., 70: 271-278.

Theodoropoulou, S., G. Spanakos, C.N. Baxevanis, M. Economou, A.D. Gritzapis, M.P. Papamichail and C.N. Stefanis, 2001. Cytokine serum levels, autologous mixed lymphocyte reaction and surface marker analysis in never medicated and chronically medicated schizophrenic patients. Schizophr. Res., 47: 13-25.

Toyooka, K., Y. Watanabe, S. Iritani, E. Shimizu and M. Iyo *et al*., 2003. A decrease in interleukin-1 receptor antagonist expression in the prefrontal cortex of schizophrenic patients. Neurosci. Res., 46: 299-307.

Viberg, H., W. Mundy and P. Eriksson, 2008. Neonatal exposure to decabrominated diphenyl ether (PBDE 209) results in changes in BDNF, CaMKII and GAP-43, biochemical substrates of neuronal survival, growth and synaptogenesis. Neurotoxicology, 29: 152-159.

Villemain, F., L. Chatenoud, A. Galinowski, F. Homo-Delarche and D. Ginestet *et al*., 1989. Aberrant T cell-mediated immunity in untreated schizophrenic patients: Deficient interleukin-2 production. Am. J. Psychiatry, 146: 609-616.

Watanabe, Y., T. Someya and H. Nawa, 2010. Cytokine hypothesis of schizophrenia pathogenesis: Evidence from human studies and animal models. Psychiat. Clin. Neurosci., 64: 217-230.

Zhang, X.Y., D.F. Zhou, P.Y. Zhang, G.Y. Wu, L.Y. Cao and Y.C. Shen, 2002. Elevated interleukin-2, interleukin-6 and interleukin-8 serum levels in neuroleptic-free schizophrenia: Association with psychopathology. Schizophr. Res., 57: 247-258.

Zhang, X.Y., D.F. Zhou, L.Y. Cao, P.Y. Zhang, G.Y. Wu and Y.C. Shen, 2004. Changes in serum interleukin-2, -6 and -8 levels before and during treatment with risperidone and haloperidol: Relationship to outcome in schizophrenia. J. Clin. Psychiat., 65: 940-947.

Zhang, X.Y., D.F. Zhou, L.Y. Qi, S. Chen and L.Y. Cao *et al*., 2009. Superoxide dismutase and cytokines in chronic patients with schizophrenia: Association with psychopathology and response to antipsychotics. Psychopharmacology, 204: 177-184.