

Effects of Antipsychotics on the Inflammatory Response System of Patients with Schizophrenia in Peripheral Blood Mononuclear Cell Cultures

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Objective: We investigated the effects of antipsychotics on immune-challenged peripheral blood mononuclear cell (PBMC) cultures.

Methods: Blood samples were collected from twelve patients with first-episode schizophrenia. The PBMCs were separated and cultures were prepared and stimulated with lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (poly(I:C)), and then separately treated with a typical antipsychotic (haloperidol) or atypical antipsychotic (clozapine, quetiapine, or risperidone). Pro-inflammatory (interferon gamma [IFN- γ]) and anti-inflammatory (interleukin [IL]-4 and IL-10) cytokine levels were measured in the LPS- or poly(I:C)-stimulated PBMC cultures treated with antipsychotics.

Results: Haloperidol and quetiapine significantly increased the IL-4 levels ($p < 0.05$) in LPS-stimulated PBMC cultures, while clozapine and quetiapine significantly enhanced the IL-4 levels ($p < 0.05$) in poly(I:C)-stimulated PBMC cultures. Only treatment with haloperidol resulted in a significant increase in IL-10 production ($p < 0.05$) in LPS-stimulated PBMC cultures, whereas clozapine, quetiapine, and risperidone treatment significantly increased IL-10 production ($p < 0.05$) in poly(I:C)-stimulated PBMC cultures. All of the antipsychotics reduced the IFN- γ level significantly ($p < 0.05$) in LPS- and poly(I:C)-stimulated PBMC cultures.

Conclusion: Antipsychotic treatment altered immune function by raising the levels of anti-inflammatory cytokines (IL-4 and IL-10) and suppressing the levels of pro-inflammatory cytokines (IFN- γ).

KEY WORDS: Schizophrenia; Cytokines; Antipsychotic; Lipopolysaccharides.

INTRODUCTION

The interaction of schizophrenia with the immune system has been investigated by various means. Previous research has associated schizophrenia with polymorphisms in genes that support immune function,¹⁾ prenatal infectious mechanisms,²⁾ disrupted cytokine networks in adults,^{3,4)} and changes in circulating peripheral immune cells.⁵⁾ In patients with schizophrenia, there are increased numbers of immune cells, which travel through the bloodstream and migrate into peripheral tissues.⁵⁾ For example, monocyte counts are higher in schizophrenic patients (SCP),^{6,7)} and studies of leukocytes have reported unchanged,^{8,9)} increased,^{10,11)} and decreased total T cell counts¹²⁾ in similar populations. In addition to T cells, elevated numbers⁷⁾ and the hyperfunction¹³⁾ of B cells have

been reported.

In SCP, alterations in cytokine concentrations,¹⁴⁾ the number of cytokine receptors,¹⁵⁾ and the amount of cytokine activity modifiers in peripheral blood and cerebrospinal fluid (CSF)¹⁵⁾ have been reported. Cytokines and their receptors predominately control communication and cross-talk between the immune and central nervous systems, but are not well-regulated in SCP compared to healthy people, particularly in first-episode cases.¹⁶⁾ Similarly, an imbalance in T-helper 1 (Th1) and T-helper 2 (Th2) immune responses in first-episode SCP has been reported.¹⁷⁾ A decreased Th1 immune response results in the diminished release of interferon gamma (IFN- γ), while an enhanced Th2 response leads to increases in interleukin (IL)-4 and IL-10 release.¹⁸⁾ In 2013, García-Bueno *et al.*¹⁹⁾ collected blood samples from 117 first-episode SCP and stimulated peripheral blood mononuclear cell (PBMC) cultures to investigate pro- and anti-inflammatory cytokine dysregulation. They found increased levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines in the cultures and suggested that the physiological balance of inflammatory pathways

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was disturbed in first-episode SCP. The authors emphasized the role of nuclear factor κ B (NF κ B) in this process and demonstrated that the activation of this factor leads to the release of pro-inflammatory components. In addition to cytokines, there are decreased concentrations of neopterin and increased concentrations of CD3+ and CD4+ cells in first-episode SCP. Moreover, there is an altered ratio of CD4+/CD8+ cells in SCP, which is more pronounced in first-episode cases.

Various cytokines have been studied in connection with schizophrenia, but the findings are inconsistent. For example, studies have shown increased levels of IL-6 and tumor necrosis factor (TNF)- α ^{8,20-23)} but decreased levels of IL-2^{24,25)} in SCP, while others have found no change^{26,27)} in cytokine levels. Moreover, there is contradictory evidence concerning anti-inflammatory cytokines such as IFN- γ , IL-4, and IL-10. Cell cultures from healthy individuals have been found to have decreased levels of IL-4²⁷⁾ or to exhibit no difference.²⁸⁾ In 1994, Inglot and co-workers²⁹⁾ suggested a connection between IFN- γ concentrations and the psychopathology of schizophrenia. They found patients with positive symptoms exhibit elevated production of IFN- γ and negative symptoms are associated with decreased IFN- γ production. In contrast, other studies have found increased levels of IFN- γ in the serum and plasma of SCP^{27,28,30)} but decreased levels of IFN- γ in whole blood cell cultures.^{9,31)} Non-paranoid SCP exhibit increased levels of IL-10 relative to healthy controls³²⁾ but other evidence does not support this finding.³³⁾ However, a strong positive correlation between the severity of negative symptoms and the concentration of IL-10 in CSF has been demonstrated. A shift from Th1 immunity to Th2 immunity has been proposed as a pathophysiological mechanism underlying schizophrenia^{34,35)} and would be indicated by a lower IFN- γ /IL-4 ratio.²⁸⁾ However, other studies have found a higher ratio in SCP. This suggests that the underlying pathology of schizophrenia involves disturbances in the balance between pro- and anti-inflammatory cytokines rather than a directional shift in the number of Th1 and Th2 cells. Alternatively, this shift could result from treatment with antipsychotics.³⁶⁾ Accordingly, it has recently been demonstrated that both typical and atypical antipsychotic drugs influence the production of cytokines.²⁰⁾

Haloperidol is a typical antipsychotic. In PBMC cultures, haloperidol has been shown to normalize increased IL-2 levels³⁷⁾ and inhibit mitogen-stimulated IL-2 production,^{38,39)} but inconsistent results have been found concerning the production of IFN- γ . Haloperidol may stim-

ulate⁴⁰⁾ or inhibit^{38,39)} IFN- γ in *in vitro* cell cultures. Moreover, haloperidol administration was shown to increase the level of IL-10 in PBMC cultures,³⁹⁾ but no changes were found in lipopolysaccharide (LPS)-treated mice.⁴¹⁾

Clozapine, risperidone, and quetiapine are common atypical antipsychotics. In 1994, the influence of clozapine on IL-2 was shown to be modulated by increases in the concentration of soluble IL-2 receptors;⁴²⁾ this finding was later confirmed by several other studies.^{43,44)} No consistent results have been found regarding the immunomodulatory effects of risperidone, but a recent study investigated the beneficial effects of risperidone on IFN- γ -stimulated microglia.⁴⁵⁾ However, risperidone has also been shown to inhibit the production of pro-inflammatory cytokines such as TNF- α and IL-6⁴⁶⁾ and to increase anti-inflammatory cytokines such as IL-10⁴¹⁾ but not affect the plasma IL-4 concentration⁴⁷⁾ in SCP. There is no clear evidence regarding the effects of quetiapine on immunomodulation in SCP, but one study reported that the drug reduced IL-2 production.⁴⁸⁾

The present study analyzed pro- and anti-inflammatory cytokine levels in cultured PBMCs from untreated first-episode SCP because this population typically produces high concentrations of cytokines. Moreover, the stimulation of PBMC cultures by LPS or polyinosinic:polycytidylic acid (poly[I:C]) leads to the release of various inflammatory cytokines and chemokines. Therefore, the immunomodulatory effects of typical (haloperidol) and atypical (clozapine, risperidone, and quetiapine) antipsychotic drugs could be observed in a situation that closely mimics natural circumstances. Typical and atypical antipsychotics were selected due to their negligible side effects and popularity for the treatment of schizophrenia. The concentrations of IFN- γ (a pro-inflammatory cytokine) and IL-4 and IL-10 (anti-inflammatory cytokines) were measured because these cytokines are consistently associated with schizophrenia.

LPS is a bacterial endotoxin found in the outer membrane of Gram-negative bacteria. It is primarily detected by its specific receptor, toll-like receptor 4 (TLR-4), leading to the production of several cytokines and chemokines. Poly(I:C) is a synthetic analog of double-stranded RNA, which is produced during the replication of RNA and DNA viruses.⁴⁹⁾ It is mainly detected by endosomally localized TLR-3 when added to culture medium,⁵⁰⁾ but the poly(I:C)-induced immune response is non-specific, meaning that it stimulates the production of inflammatory cytokines rather than particular anti-viral antibodies. LPS

and poly(I:C) are a cost-effective means of inducing the short-term stimulation of PBMC cultures, which can be used to analyze the cytokine profile of schizophrenics. The effects of poly(I:C) last for approximately 48 hours.

METHODS

Subjects

Blood samples were collected from 12 first-episode SCP (6 females, 6 males; age range, 19-62 years; mean age, 34.08±13.39 years) to analyze the production of cytokines. All subjects provided written consent and the experimental procedure was previously approved by the ethics committee of the Department of Pharmacy at North South University in Dhaka, Bangladesh. Subjects were excluded on the basis of the following criteria: a) a past or present history of psychiatric disorders, b) use of major psychotropic medications such as antidepressants and antipsychotics, c) drug and/or alcohol abuse or dependence, d) any medical (e.g., endocrine, immune, or metabolic) disorder such as diabetes, autoimmune disorders, inflammatory bowel disease, or acquired immunodeficiency syndrome, or e) current (2 weeks prior to the first blood sample) diagnosis of an infectious, allergic, or inflammatory response. The subjects abstained from caffeine, alcohol, and nicotine for at least 8 hours prior to blood sampling and were asked to fast overnight.

Blood Collection, PBMC Separation, and Culture Preparation

Venous blood (18 ml) from first-episode SCP was collected in heparinized tubes at approximately 8:00 AM. The samples were diluted (1:1) with sterile phosphate-buffered saline, layered over Ficoll-Hypaque (GE Healthcare, Little Chalfont, UK), and centrifuged at 1,500 rpm for 30 minutes at room temperature. The interphase layer was withdrawn and the isolated PBMCs were incubated in RPMI medium-1640 (R-8005; Sigma, St. Louis, MO, USA) containing 1% penicillin (Sigma) with L-glutamine and Phenol Red in microtitration plates at a concentration of 10^6 cells per well. The samples were incubated for 72 hours in a humidified atmosphere at 37°C with 5% CO₂ to obtain peak cumulative responses for most cytokines. The plates were centrifuged at 1,500 rpm for 8 minutes following incubation. The supernatants were carefully removed under sterile conditions, divided into Eppendorf tubes (Eppendorf India Ltd., Dattawadi, India), and immediately frozen at -70°C until thawed for assay.

Immune Challenge and the Addition of Antipsychotic Medications

LPS and poly(I:C) (1 mg/ml) were added to PBMC cultures for bacterial and viral stimulation, respectively. In the untreated antipsychotic condition, 75 μ l of PBMC culture was combined with 225 μ l of stimulant medium (LPS or poly[I:C]) for a final volume of 300 μ l. The final volume was placed in 24-well sterile plates. In the antipsychotic treatment condition, 10 μ l of antipsychotic drug (haloperidol, clozapine, quetiapine, or risperidone) and 75 μ l of PBMC culture was combined with 215 μ l of stimulant medium (LPS or poly[I:C]) for a final volume 300 μ l. All antipsychotic drugs were obtained from a local pharmaceutical company in Bangladesh and given in concentrations of 1 mg/ml. The cytokine (IL-4, IL-10, and IFN- γ) concentrations in the PBMC culture supernatants were quantified using enzyme-linked immunosorbent assays. The intra-assay coefficient of variation was less than 8%, and the limits of detection were as follows: IL-10, 10 pg/ml; IFN- γ , 1.03 pg/ml; and IL-4, 0.39 pg/ml.

Statistical Analysis

To investigate the effects of antipsychotic drugs on cytokine (IL-4, IL-10, and IFN- γ) levels in immune-stimulated PBMC cultures, a repeated measure analysis of variance (ANOVA) was performed. The ANOVA was used to analyze: 1) within-subject variability associated with the effects of antipsychotic drugs and/or the effects of LPS or poly(I:C) treatment as a temporal condition, and 2) between-subject variability with sex as a factor. Differences were considered significant at $p < 0.05$. All data are presented as mean±standard error of the mean. SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA) was used in all analyses.

RESULTS

Effect of Antipsychotics on the IL-4 Level in LPS- and Poly(I:C)-stimulated PBMC Cultures

Both haloperidol ($F[1, 9]=7.98, p < 0.05$) and quetiapine ($F[1, 9]=7.86, p < 0.05$) significantly increased the level of IL-4 in LPS-stimulated PBMC cultures (Fig. 1A). In the poly(I:C)-stimulated PBMC cultures, clozapine ($F[1, 9]=13.89, p < 0.05$) and quetiapine ($F[1, 9]=6.73, p < 0.05$) significantly enhanced the level of IL-4 (Fig. 1B).

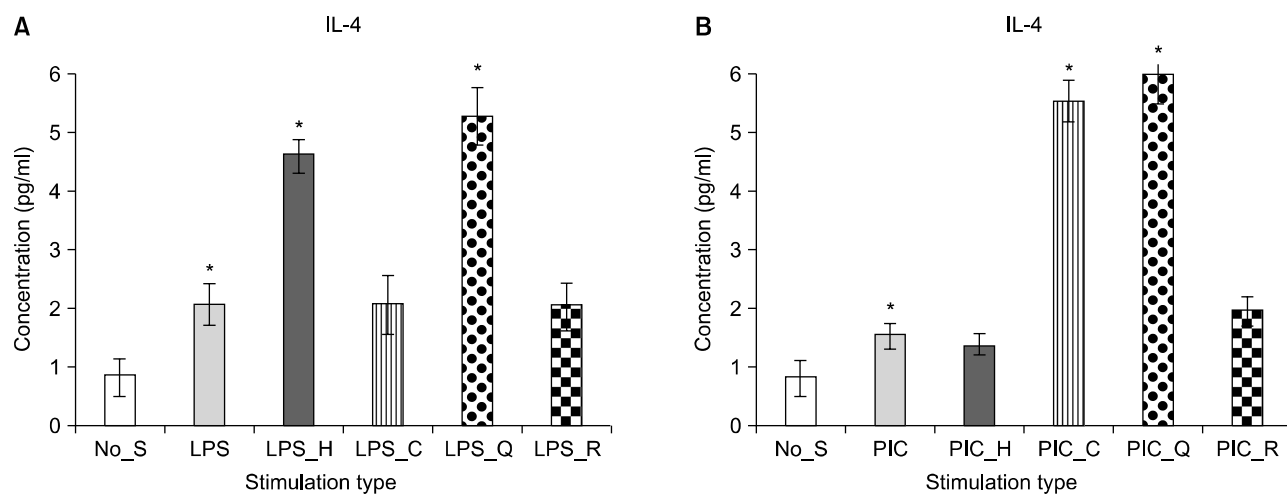


Fig. 1. Mean value of interleukin 4 (IL-4) level in the peripheral blood mononuclear cell culture. Stimulation by LPS (A) and stimulation by PIC (B). No_S, no stimulation; LPS, lipopolysaccharide; PIC, polyinosinic:polycytidylic acid; H, haloperidol; C, clozapine; Q, quetiapine; R, risperidone. * $p < 0.05$; mean \pm standard error of mean.

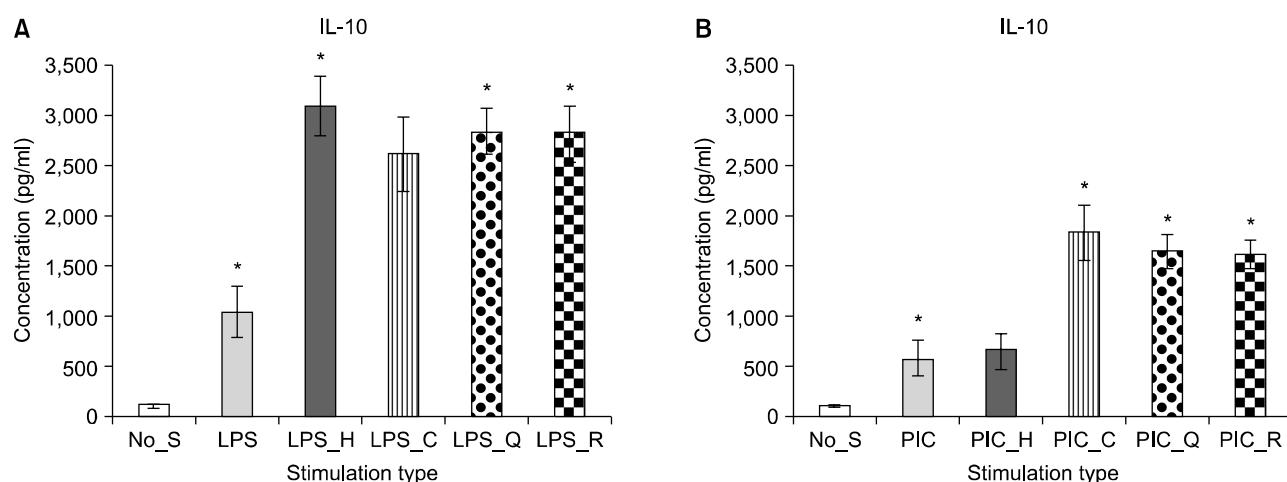


Fig. 2. Mean value of interleukin 10 (IL-10) level in the peripheral blood mononuclear cell culture. Stimulation by LPS (A) and stimulation by PIC (B). No_S, no stimulation; LPS, lipopolysaccharide; PIC, polyinosinic:polycytidylic acid; H, haloperidol; C, clozapine; Q, quetiapine; R, risperidone. * $p < 0.05$; mean \pm standard error of mean.

Effect of Antipsychotics on the IL-10 Level in LPS- and Poly(I:C)-stimulated PBMC Cultures

Haloperidol ($F[1, 9]=5.37, p < 0.05$), quetiapine ($F[1, 9]=5.32, p < 0.05$), and risperidone ($F[1, 9]=4.68, p < 0.05$) treatment significantly increased the production of IL-10 in the LPS-stimulated PBMC cultures (Fig. 2A). In the poly(I:C)-stimulated PBMC cultures, clozapine ($F[1, 9]=12.68, p < 0.05$), quetiapine ($F[1, 9]=11.55, p < 0.05$), and risperidone ($F[1, 9]=54.9, p < 0.05$) treatment resulted in significantly enhanced IL-10 production (Fig. 2B).

Effect of Antipsychotics on the IFN- γ Level in LPS- and poly(I:C)-stimulated PBMC Cultures

Haloperidol ($F[1, 9]=20.06, p=0.002$), clozapine ($F[1, 9]=29.40, p < 0.05$), quetiapine ($F[1, 9]=14.98, p < 0.05$), and risperidone ($F[1, 9]=23.33, p < 0.05$) significantly reduced the production of IFN- γ in the LPS-stimulated PBMC cultures. Likewise, in the poly(I:C)-stimulated PBMC cultures, haloperidol ($F[1, 9]=44.45, p < 0.05$), clozapine ($F[1, 9]=16.93, p < 0.05$), quetiapine ($F[1, 9]=65.04, p < 0.05$), and risperidone ($F[1, 9]=10.28, p < 0.05$) significantly reduced the production of IFN- γ (Fig. 3).

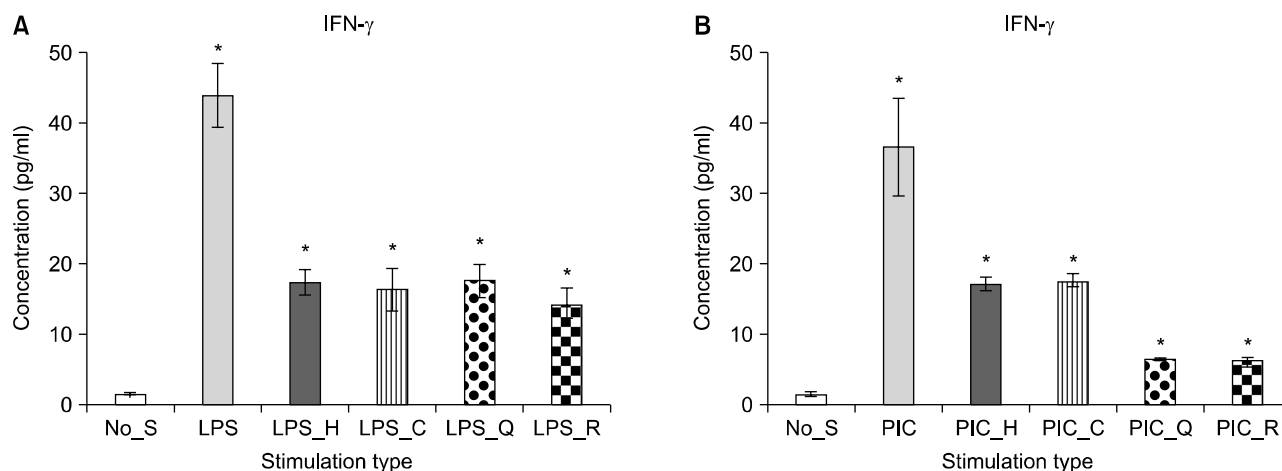


Fig. 3. Mean value of interferon gamma (IFN- γ) level in the peripheral blood mononuclear cell culture. Stimulation by LPS (A) and stimulation by PIC (B). No_S, no stimulation; LPS, lipopolysaccharide; PIC, polyinosinic:polycytidylic acid; H, haloperidol; C, clozapine; Q, quetiapine; R, risperidone. * $p < 0.05$; mean \pm standard error of mean.

DISCUSSION

The stimulation of cultured PBMCs from first-episode SCP by LPS and poly(I:C) resulted in the altered production of pro- and anti-inflammatory cytokines. Additionally, cytokine levels were affected by treatment with antipsychotics. The use of cultured PBMCs may better reflect the inflammatory process than the assessment of serum cytokine levels. The production of IFN- γ , IL-4, and IL-10 have not been reported by previous studies of PBMC cultures from first-episode SCP. LPS and poly(I:C) are agents that stimulate immune cells. The stimulation of PBMCs by LPS leads to binding with TLR-4 and the subsequent release of cytokines and other inflammatory mediators, whereas stimulation by poly(I:C) leads to binding with TLR-3 and cytokine release. LPS and poly(I:C) both initiate nuclear localization of the transcription factor NF κ B and the subsequent activation of genes in pro-inflammatory pathways. Two important conclusions may be drawn from this study. First, antipsychotics enhance the production of anti-inflammatory cytokines (IL-4 and IL-10) in LPS- and poly(I:C)-stimulated PBMC cultures, and, second, antipsychotics reduce pro-inflammatory cytokines (IFN- γ) in LPS- and poly(I:C)-stimulated PBMC cultures.

The findings of the present study are consistent with those of previous studies. For example, increased levels of IL-10 in cultured PBMCs have been found following haloperidol treatment,³⁹⁾ and a robust increase in IL-10 serum levels induced by clozapine has been reported.⁴¹⁾ There are no consistent findings regarding the immunomodulatory effects of risperidone. Some studies found that

risperidone decreased plasma levels of IFN- γ ,^{37,46,47)} while other studies reported no differences.^{37,47)} A recent study investigating the beneficial effects of risperidone on IFN- γ -stimulated microglia found that it inhibits the production of nitric oxide (NO) and other pro-inflammatory cytokines, including IFN- γ and IL-6.⁴⁵⁾ Furthermore, increased levels of IL-10 and unchanged levels of IL-4 have been observed in the plasma of SCP.⁴⁷⁾ The treatment of *in vitro* cell cultures has been shown to either stimulate⁴⁰⁾ or inhibit^{38,39)} IFN- γ , and a recent study reported a weak effect on the inhibition of harmful NO by IFN- γ -activated microglia.⁴⁵⁾ Haloperidol seems to normalize increased IL-2 plasma and serum levels³⁷⁾ and inhibit mitogen-stimulated IL-2 production in PBMCs.^{38,39)} The effects of haloperidol are particularly evident in patients with positive symptoms.⁵¹⁾ Haloperidol lowers TNF- α production in LPS-stimulated monocytes,⁵²⁾ but does not affect the IL-6 concentration in serum, plasma,^{37,53,54)} or CSF⁵⁵⁾ from SCP.

In this study, only one concentration was used for all antipsychotic drugs. This concentration was identified by a review of previous literature⁵⁶⁾ and was chosen on the basis of bioavailability following oral administration. Different concentrations of antipsychotic drugs should be employed in future trials to better evaluate changes in the levels of anti-inflammatory cytokines in stimulated PBMC cultures.

An important consideration of this study was to not include healthy volunteers because this population is not as vulnerable to changes in the cytokine system following LPS and poly(I:C) stimulation as are first-episode SCP. However, we previously conducted an experiment in

which PBMC cultures were prepared from the blood of healthy volunteers and the same cytokines (IFN- γ , IL-4, and IL-10) were measured.⁵⁷⁾ Several major differences were observed between the PBMC cultures of healthy subjects and first-episode SCP. First, LPS and poly(I:C) caused an increase in pro-inflammatory cytokine levels and a decrease in anti-inflammatory cytokine levels (IL-4 and IL-10) in first-episode SCP compared to healthy volunteers, consistent with previous studies. Second, both typical and atypical antipsychotics alter immune function via the suppression of pro-inflammatory cytokine levels (IFN- γ) and the elevation of anti-inflammatory cytokines (IL-10).⁵⁷⁾ Third, antipsychotics do not influence IL-4 concentrations in healthy volunteers. In first-episode SCP, however, antipsychotics cause an increase in IL-4 release.

There are a number of limitations to this study. First, the present study utilized a small sample size. A larger number of patient samples would provide more opportunity to analyze the parameters of this group. Second, only three inflammatory cytokines were considered because this study was backed by personal finance. TNF- α , IL-2, IL-6, and other important inflammatory cytokines should also be analyzed to better explore the consequences of first-episode schizophrenia. Third, the limited resources and facilities of research laboratories in our country make it very challenging to conduct this type of cell culture research. Finally, in some instances, the IL-4 levels were not similarly increased in LPS- and poly(I:C)-treated cultures. This may be due to the short half-life of cytokines. In conjunction with previous findings, the present results suggest that antipsychotics have a positive influence on pro- and anti-inflammatory cytokines in first-episode SCP.

The role of the cytokine system in the pathogenesis of schizophrenia is well-established. Here, we demonstrated that antipsychotic agents might provide benefits in terms of the imbalance of peripheral pro- and anti-inflammatory cytokine levels in schizophrenic subjects. These data should be confirmed through an analysis of a large number of first-episode SCP and the measurement of additional cytokines.

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