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More dampened monocytic Toll-like receptor 4 response to lipopolysaccharide and its association with cognitive function in Chinese Han first-episode patients with schizophrenia

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ABSTRACT

Objective: Accumulating evidence suggests alterations of the innate immune system are related to schizophrenia, although the precise mechanism remains to be elucidated. In this study, we aimed to detect the monocytic toll-like receptor 4 (TLR4) expression under basal and lipopolysaccharide (LPS)-stimulated conditions in first-episode (FE) Han Chinese patients with schizophrenia, as well as its association with cognitive function.
Methods: Whole blood samples were taken in 42 FE schizophrenia patients and 36 healthy controls. Expressions of TLR4 on monocytes under basal and LPS-stimulated conditions were measured with flow cytometry. Psychopathological symptoms of schizophrenia were assessed by the Positive and Negative Syndrome Scale (PANSS) and the MATRICS Consensus Cognitive Battery (MCCB) was administered to all of the participants.
Results: We found no differences in percentage and mean fluorescence intensity (MFI) of TLR4 expression on monocytes between patients and controls at basal status. However, LPS challenge resulted in a lower cell-surface level of TLR4 on monocytes in FE schizophrenia patients as compared to healthy controls (TLR4+%: $F = 4.092$, $p = 0.047$; TLR4 + MFI: $F = 4.820$, $p = 0.031$). In addition, correlation analysis together with multivariate linear regression analysis identified basal percentage of TLR4 in monocytes as the beneficial factor for visual learning and working memory in FE patients with schizophrenia.
Conclusions: Our findings suggested that TLR4 may be involved in the pathophysiology of schizophrenia, corroborating the role of innate immunity-related functional deficits in increased risk of schizophrenia.

1. Background

Researches in recent years have indicated complex interactions between the innate immune system and the brain, which may be implicated in the pathophysiology of schizophrenia. The innate immunity is the first line of host defense against pathogens, and its reaction is regarded as rapid but non-specific compared to the adaptive immunity (Iwasaki and Medzhitov, 2015). Toll-like receptors (TLRs), as pivotal sensors of the innate immune system, have been identified as key host molecules required for recognizing damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) to induce innate immune responses (Kawai and Akira, 2010). Recent genomic and proteomic studies have indicated that disrupted TLR signaling pathway may increase the susceptibility to schizophrenia (Crisafulli et al., 2015; Yu et al., 2014).

TLR4, also known as CD284, is the most studied member in the TLRs family. It is a type I transmembrane glycoprotein mainly expressed on monocytes, macrophages, granulocytes, dendritic cells and endothelial cells (Kawai and Akira, 2010; Lucas and Maes, 2013). Notably, microglia as the major resident mononuclear phagocytes in the central nervous system (CNS) parenchyma, also express abundantly TLR4 (Trotta et al., 2014). TLR4 recognizes host-derived DAMPs released from injured cells, such as oxidized low density lipoprotein (OxLDL), high mobility

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group protein 1 (HMGB1), heat shock proteins (HSPs) and substance P, among others. Additionally, TLR4 recognizes a great variety of PAMPs, such as lipopolysaccharide (LPS) from gram-negative bacteria that is also known as bacterial endotoxin. LPS-induced activation of TLR4 triggers the translocation of nuclear factor-kappa B (NF-κB) to karyon and subsequent production of pro-inflammatory cytokines, such as interleukin-6 (IL)-6, tumor necrosis factor alpha (TNF)-α, as well as IL-1β, to eradicate infection (Lucas and Maes, 2013).

Some authors have proposed that inflammatory consequences of TLR4 activation may contribute to the neuropathological changes of schizophrenia and therefore modulating TLR4 could be a promising therapeutic approach for schizophrenia (Garcia Bueno et al., 2016; Lucas and Maes, 2013; Venkatasubramanian and Debnath, 2013). This speculation is corroborated by the following observations in animal research. Firstly, TLR4 may play an important role in synaptic plasticity and neurodevelopment (Okun et al., 2011), and TLR4 may interfere with multiple neurotransmission pathways, including the dopaminergic, serotonergic, glutamatergic and GABAergic systems (Garcia Bueno et al., 2016). Secondly, TLR4 may participate in maternal immune activation caused by infection or stress, which has been regarded as a significant primer for schizophrenia (Estes and McAllister, 2016; Venkatasubramanian and Debnath, 2013). Finally, LPS can induce schizophrenia-like behavior *via* intrahippocampal injection during the neonatal period (Zhu et al., 2014), whereas inhibiting TLR4 activation by an antagonist TAK-242 or peptidic blockers can decrease neuroinflammation in the brain and suppress the induction of sickness behaviors (Garate et al., 2014; Hines et al., 2013). Similarly, in human research, Borja García-Bueno et al. recently confirmed that TLR4 and NF-κB protein levels were higher in the postmortem prefrontal cortex from chronic schizophrenia patients than matched controls (Garcia-Bueno et al., 2016). In addition, positron emission tomography imaging studies (Ottoy et al., 2018; van Berckel et al., 2008) have demonstrated that activation of microglia plays an important role in the pathogenesis of schizophrenia, *via* presumably the TLR4/NF-κB inflammatory signaling pathway (Hines et al., 2013; Zhu et al., 2014).

Parallel to brain microglia, monocytes are the major myeloid innate immune cells in the peripheral blood, and both cell types come from myeloid precursors cells that share the overlapping albeit not exactly the same hematopoietic origin (Ginhoux et al., 2010). Also, studies suggest that monocyte-derived macrophages can infiltrate into the brain and become microglia under pathological conditions (Ginhoux et al., 2013; Yamasaki et al., 2014). It is considered that peripheral blood leucocytes may be a useful surrogate for patterns of DNA methylation and gene expression in the CNS, particularly in the aspects of encoding cytokines, hormones and growth factors (Sullivan et al., 2006; Tylee et al., 2013). Furthermore, proinflammatory cytokines derived from monocytes and macrophages, such as IL-1β, IL-6 and TNF-α, can communicate with the brain through humoral, neural and endocrinal pathways, which may exert a profound influence on cognition, mood and behavior (Khandaker and Dantzer, 2016).

However, current researches on the relationship between monocytes along with its pivotal pattern recognition receptor TLR4 and schizophrenia remain limited. So far, only three studies have raised a concern in this respect (Keri et al., 2017a, b; Muller et al., 2012). These studies provided evidence that schizophrenia patients exhibited an increased TLR4 expression on monocytes relative to healthy controls (Keri et al., 2017a, b; Muller et al., 2012), whereas stimulation of monocytic TLR4 by LPS resulted in weaker intracellular pro-inflammatory cytokine production in schizophrenia patients than in control subjects (Keri et al., 2017b; Muller et al., 2012), reflecting a blunted monocytic activation in schizophrenia and suggesting increased TLR4 expression as a compensatory mechanism for its functional deficit (Muller et al., 2012). However, no significant association between psychopathology and TLR4 expression on monocytes in schizophrenia patients was found in previous studies (Keri et al., 2017a; Muller et al., 2012). Although a higher percentage of TLR4+ monocytes at the basal level was found to be related to more severe cognitive deficits (Keri et al., 2017a), the relevance of TLR4 activation in response to LPS challenge to cognition in schizophrenia has not been previously addressed.

Taken together, in the present study, we intended to a) detect the TLR4 expression on monocytes under basal and LPS-stimulated conditions in first-episode (FE) Han Chinese patients with schizophrenia and in healthy controls, and b) examine its relationship with clinical symptoms measured by the Positive and Negative Syndrome Scale (PANSS) as well as with neurocognitive functions assessed by the MATRICS Consensus Cognitive Battery (MCCB).

2. Materials and methods

2.1. Participants

This study was conducted according to the Declaration of Helsinki and was approved by the Medical Ethical Committee of Beijing Huilongguan Hospital, and written informed consent was obtained from all participants before the initiation of study procedures. A detailed medical history was obtained from patients and the inclusion criteria were: 1) Structured Clinical Interview for DSM-IV (SCID) administered by two experienced psychiatrists confirming the diagnosis of schizophrenia or schizophreniform disorder; 2) Han nationality and age of 16–45 years old; 3) clinical course less than 2 years; 4) a cumulative psychotropic drugs exposure ≤14 days; 5) years of schooling >8 years; 6) physically healthy; 7) without any immunomodulator, immunosuppressant or anti-inflammatory treatments in the last 6 months; 8) no substance and alcohol abuse or dependence; 9) without electric convulsive therapy or transcranial magnetic stimulation in the past 12 months.

Healthy controls of the Chinese Han population were recruited from the local community. Medical history, physical examination, laboratory tests, current mental status and family history of any mental illness were assessed by a psychiatrist. Subjects with abnormal condition of the above criteria were excluded. None of the normal controls was treated with immune-related medication in the previous 6 months.

2.2. Blood sampling and expression of TLR4 on peripheral blood monocytes assessed by flow cytometry

All participants fasted for 10 h before blood drawing. Five (5) ml of peripheral blood was collected in heparin lithium-anticoagulant tube and processed within half an hour after blood drawing. Whole blood samples (100 μl) were transferred into polystyrene FACS tubes and stained with 10 μl FITC-labeled mouse anti-human CD14 (Clone M5E2; Catalog Number 555397; BD Biosciences) and 3.5 μl PE-labeled mouse anti-human TLR4 (CD284) (Clone TF901; Catalog Number 564215; BD Biosciences). After 15 min in the dark at room temperature, 2 ml of diluted lysing solution (BD FACS™) was added to lyse red blood cells for additional 10 min. Subsequently, the samples were centrifuged at 500*g* for 5 min and the supernatants were discarded. Cell pellets were washed twice with 2 ml sheath fluid (BD FACSFlow™) and resuspended in 500 μl Dulbecco's phosphate buffered saline (DPBS), which was immediately acquisited by a BD FACSCalibur flow cytometer. To detect staining specificity, corresponding samples were stained with PE-labeled mouse IgG1, κ isotype antibodies (Clone MOPC-21; Catalog Number 554680; BD Biosciences) as negative controls.

For LPS challenge, another 100 μl of whole blood samples were stimulated with LPS (100 ng/ml, *Escherichia coli* O55:B5, SIGMA-Aldrich) at 37 °C for 5 h. Subsequent operation processes were the same as described above, including the isotype controls. Corresponding blood samples without LPS stimulation were also handled in parallel as controls.

A total of 2500 CD14+ monocytes were acquired for each sample and data were analyzed with the FlowJo V10 software. Percentage and mean fluorescence intensity (MFI) of TLR4 among CD14+ monocytes are presented. The gating strategy is shown in Fig. 1.

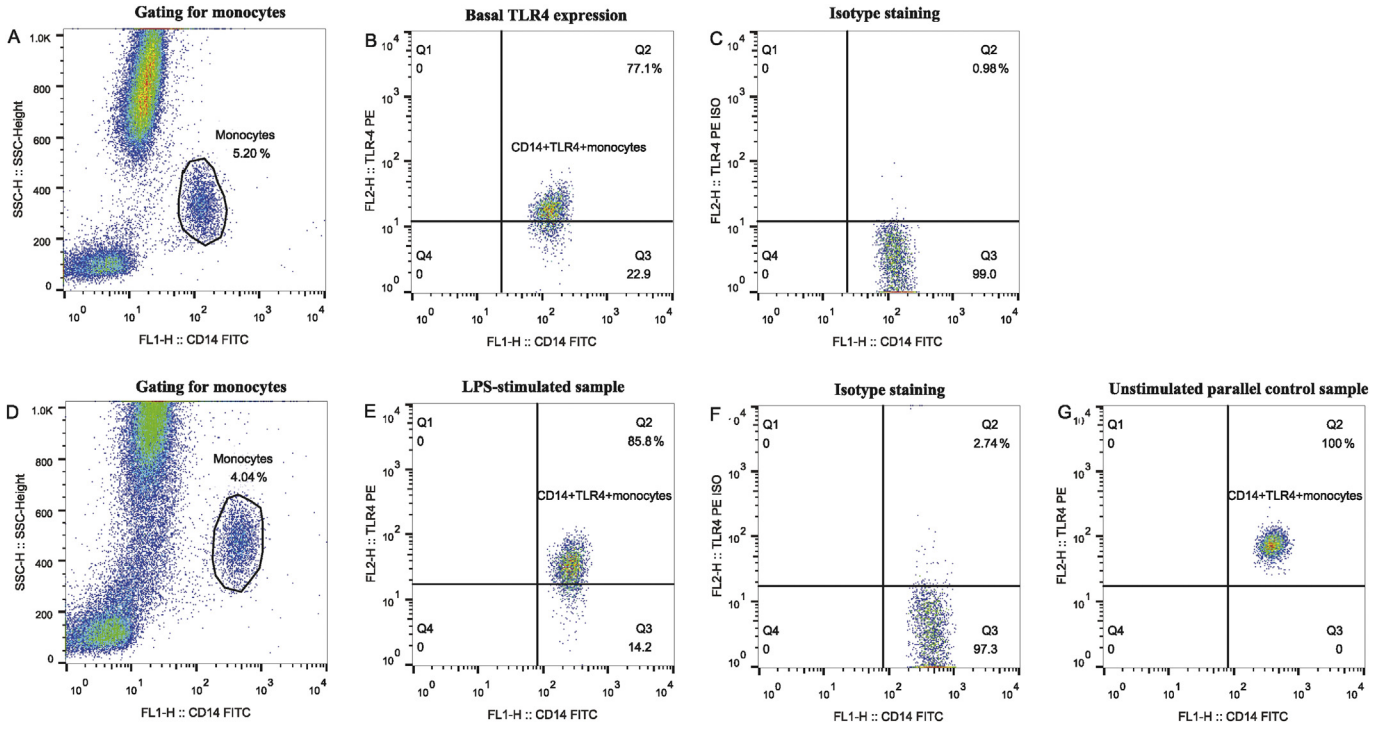

Fig. 1. Expression of Toll-like receptor 4 (TLR4) on peripheral blood monocytes determined by flow cytometry. (A–C) indicate the basal TLR4 expression on monocytes. (A) Gating for peripheral blood monocytes according to CD14+ cell populations. (B) Whole blood sample was stained with PE-labeled mouse anti-human TLR4 to assess the TLR4 surface expression, which was quantified by percentage and mean fluorescence intensity (MFI). (C) To detect staining specificity, corresponding sample was stained with PE-labeled mouse IgG1, κ isotype antibodies as negative control. (D–G) indicate the lipopolysaccharide (LPS)-stimulated experiment *in vitro* at 37 °C for 5 h. (D) Gating for peripheral blood monocytes according to CD14+ cell populations. (E) Whole blood sample was stimulated with LPS (100 ng/ml) at 37 °C for 5 h. (F) Isotype control as described above. (G) Corresponding blood sample without LPS stimulation was incubated in parallel as control. Coordinate axis represents the fluorescence intensity.

2.3. Psychopathological symptoms and cognitive assessments

PANSS was administered to schizophrenia patients on the same day of blood sampling. Before the study began, two senior psychiatrists were trained for the PANSS to ensure consistency and reliability of ratings across the study. Intra-class correlation coefficient >0.8 was maintained for the PANSS total scores after training.

Cognitive function was assessed by the Chinese version of MCCB whose validity and reliability had been previously ascertained (Zou et al., 2009). The MCCB was administered to all the participants by one trained clinical neuropsychologist within 72 h after blood drawing. As described in our previous report (Chen et al., 2017), the MCCB includes ten tests encompassing seven cognitive domains and domain T-scores as well as a composite T-score were obtained employing the MCCB scoring program.

2.4. Statistical analysis

Data were analyzed using the IBM SPSS Statistics 21.0. Comparisons between the FE patients and healthy controls were assessed with Student's *t*-test for normally distributed continuous variables, Mann–Whitney *U* test for non-normally distributed variables, and Chi-Square test for categorical data. Analysis of covariance (ANCOVA) was performed to compare the MCCB scores and the TLR4 expression on monocytes following LPS stimulation between patients and healthy subjects, using education years and unstimulated parallel control data as covariates, respectively. Relationships between variables were analyzed with Pearson product moment correlation for normally distributed data or Spearman rank correlation for non-normally distributed variables. Bonferroni corrections were applied to each test to adjust for multiple testing. Finally, we used stepwise multiple linear regression analysis to investigate the impact of TLR4 expression in monocytes on the composite and seven domains of MCCB scores respectively in both patients and controls, controlling for age, gender and education as independent covariates. All *p*-values were two-tailed and significance levels were set at 0.05.

3. Results

3.1. Demographics and clinical data

A total of 42 FE patients with schizophrenia and 36 healthy controls were recruited according to the inclusion and exclusion criteria. The patients had a mean duration of illness of 16.1 (S.D. = 5.4) months before recruitment. The patient group consisted of 5 patients who had never been exposed to antipsychotics and 37 patients who had received <10 days of antipsychotic medications (median 3 days; mean 3 days). The average of daily dosage of chlorpromazine-equivalent antipsychotic drugs was 318.9 (S.D. = 140.6) mg (risperidone, $n = 20$; olanzapine, $n = 9$; aripiprazole, $n = 7$; haloperidol, $n = 1$).

Blood hemogram indexes were within normal ranges in all participants. The FE patients did not differ from healthy volunteers with regard to demographic characteristics (age, sex ratio, and smoking status), weight characteristics (BMI and waist-to-hip ratio) or biochemical indices (serum levels of cholesterol, triglycerides, glucose and high-sensitivity C-reactive protein) (all $p > 0.05$). However, the patients had significantly lower educational levels as compared with healthy controls ($p < 0.01$, Table 1).

3.2. Comparison of TLR4 expression on CD14+ monocytes between FE patients and healthy participants

We found no differences in percentage and MFI of TLR4 expression on monocytes between patients and controls at basal status (all $p >$ 0.05, Table 2).

Table 1
Demographic and clinical characteristics of FEDN schizophrenia patients and controls.

	Schizophrenia ($n = 42$)	Healthy controls (n = 36)	t/z/x²Value	p-Value
Age (years)+[a]	25.21 ± 6.20	26.47 ± 4.40	−1.253	0.210
Gender (male/female)+[b]	18/24	21/15	1.857	0.173
Education (years)[c]	12.22 ± 2.76	14.17 ± 2.10	−3.503	0.001
Smoker/Non-smoker[b]	3/39	6/30	0.916	0.339
BMI (kg/m²)[c]	21.55 ± 3.25	21.83 ± 2.58	−0.416	0.679
Waist-to-hip ratio[a]	0.85 ± 0.06	0.85 ± 0.08	0.605	0.545
Total cholesterol (mmol/L)[c]	4.13 ± 0.84	4.26 ± 0.74	−0.731	0.467
Triglycerides (mmol/L)[a]	0.87 ± 0.36	0.95 ± 0.41	−0.957	0.339
Glucose (mmol/L)[c]	4.89 ± 0.81	5.07 ± 0.38	−1.269	0.210
hs-CRP (mg/L)[a]	1.79 ± 2.41	1.19 ± 1.97	0.817	0.414
PANSS total	80.43 ± 12.51	NA	NA	NA
P subscore	22.18 ± 5.97	NA	NA	NA
N subscore	18.78 ± 6.53	NA	NA	NA
G subscore	39.47 ± 7.37	NA	NA	NA

Data are shown as mean ± SD unless otherwise indicated.
[a] Mann–Whitney *U* test.
[b] Chi-square test.
[c] Student's *t*-test.

Similar as the basal TLR4 levels, in control samples without 5 h of LPS stimulation, expression of TLR4 on monocytes in the two groups did not differ significantly (percentage and MFI all $p > 0.1$). Compared to unstimulated parallel control samples, LPS-stimulated samples from both FE patients (TLR4 + %: 99.15 ± 1.26% *vs* 76.33 ± 12.56%, $t = 12.174$, $p < 0.001$; TLR4 + MFI: 58.13 ± 10.64 *vs* 33.94 ± 3.89, $t = 17.464$, $p < 0.001$) and healthy controls (TLR4 + %: 99.10 ± 2.23% *vs* 81.34 ± 12.45%, $t = 9.277$, $p < 0.001$; TLR4 + MFI: 60.27 ± 10.09 *vs* 36.14 ± 4.46, $t = 18.005$, $p < 0.001$) showed significant decreases of TLR4 expression on monocytes. Moreover, LPS-stimulated patient samples showed lower TLR4 expression as compared to stimulated control subjects (TLR4 + %: 76.33 ± 12.56% *vs* 81.34 ± 12.45%, $F = 4.092$, $p = 0.047$; TLR4 + MFI: 33.94 ± 3.89 *vs* 36.14 ± 4.46, $F = 4.820$, $p = 0.031$), when unstimulated parallel control data were included as covariates (Fig. 2).

We further used the formula: (unstimulated − stimulated)/unstimulated, to determine the reduced rate after stimulation with LPS. There were no significant differences in the reduced rates of neither percentage (23 ± 12% *vs* 18 ± 12%, $p = 0.053$) nor MFI (40 ± 9% *vs* 39 ± 8%, $p = 0.492$) of TLR4+ monocytes between patients and healthy controls.

There were no associations between the absolute values of TLR4 measures and current psychopathology parameters in the patient group (all $p > 0.05$). Demographic data on gender, age, educational level, BMI, waist-to-hip ratio, smoking status and biochemical indices were not associated with TLR4 expression under either basal or LPS-stimulated condition in both groups (all $p > 0.05$).

3.3. Relationship between TLR4 expression on monocytes and cognitive functioning in FEDN schizophrenia patients and healthy controls

The MCCB composite T-score and seven domain T-scores for patients and healthy controls are shown in Table 3. ANCOVA indicated significantly lower values of the composite MCCB score and all of its subscale scores in patients than normal controls (all $p < 0.01$), as expected.

Intriguingly, positive correlations between TLR4+ monocyte measures and cognitive scores were observed. In the patient group, correlation analysis revealed that the basal percentage of TLR4+ monocytes was positively associated with verbal learning ($r = 0.385$, $p = 0.012$), visual learning ($r = 0.469$, $p = 0.002$) and working memory ($r = 0.426$, $p = 0.005$). In addition, the basal MFI of TLR4+ monocytes was correlated positively with the MCCB composite score ($r = 0.426$, $p = 0.01$), visual learning ($r = 0.333$, $p = 0.047$) and processing speed ($r = 0.349$, $p = 0.037$) in the control group. However, only the association of the basal percentage of TLR4+ monocytes with visual learning and working memory passed Bonferroni test (Fig. 3). All the other associations between TLR4 measures and cognitive parameters were insignificant within either group (all $p > 0.05$).

Considering the MCCB composite score and seven subscores as the dependent variables, and the basal percentage of TLR4 on monocytes, age, gender and education as the independent variables, a stepwise multivariate linear regression model identified the basal percentage of TLR4+ monocytes as the influencing factor for the visual learning ($\beta = 0.458$, $t = 3.218$, $p = 0.003$) and working memory ($\beta = 0.367$, $t = 2.533$, $p = 0.016$) in FE patients. Nevertheless, in the linear regression analysis for associations with the MCCB composite score, visual learning and processing speed, the basal MFI of TLR4+ monocytes was not an independent contributor in healthy volunteers.

4. Discussion

The main findings of the present study include that (1) LPS challenge resulted in a lower cell-surface level of TLR4 on monocytes in Han Chinese patients with FE schizophrenia as compared to healthy controls, although there was no significant difference in basal TLR4 level between the two groups; (2) the basal percentage of TLR4+ monocytes seems beneficial to visual learning and working memory in patients with schizophrenia.

Compared to the lack of basal level difference in monocytic TLR4 expression between patients and healthy subjects, the monocytic TLR4 level was significantly lower in patients than healthy subjects after LPS stimulation. This demonstrates a weakened TLR4 activation in monocytes in FE schizophrenia. In agreement, several published lines of evidence have shown that stimulation of TLR4 leads to less increased production of monocytic proinflammatory cytokines in schizophrenia patients relative to counterparts from healthy controls, including IL-1β, IL-6 and TNF-α (Keri et al., 2017b; Muller et al., 2012). Suppressed TLR4 signal transduction in peripheral blood monocytes of patients with schizophrenia may result in a reduced clearance of pathogens from the body and persistent low-grade inflammation (Muller et al., 2012). Indeed, a large-scale national cohort study from Denmark identified an association between a wide range of infections and subsequent risk for developing schizophrenia, with exposure to bacterial infection carrying the highest risk of schizophrenia (Nielsen et al., 2014).

It is noteworthy that TLR4 signaling is a sufficient but not exclusive pathway to modulate monocytic activity in monocytes. For instance, Kéri et al. demonstrated that stimulation of TLR4 and neuregulin 1 (NRG1) receptor ErbB on monocytes induced opposite proinflammatory cytokines responses in first-episode, drug-naïve patients with schizophrenia, namely, NRG1-induced activation of ErbB resulted in more enhanced production of proinflammatory cytokines in patient group (Keri et al., 2017b). Furthermore, some studies have shown over-activation of circulating monocytes in schizophrenia, as manifested by the ultrastructural abnormalities of monocytes, including expanded area of nucleolus, mitochondria and lysosomes (Uranova et al., 2017), as well as elevated expression of immune genes in monocytes

Table 2
Basal TLR4 expression on CD14+ monocytes in FEDN schizophrenia patients and healthy controls.

CD14 + TLR4+ expression	Schizophrenia (n = 42)	Healthy controls ($n = 36$)	z Value	p-Value
TLR4+ monocytes (%)	80.02 ± 10.05	82.51 ± 8.64	−1.181	0.238
TLR4+ monocytes (MFI)	17.19 ± 3.97	18.38 ± 3.84	−1.332	0.183

Data are shown as mean ± SD.

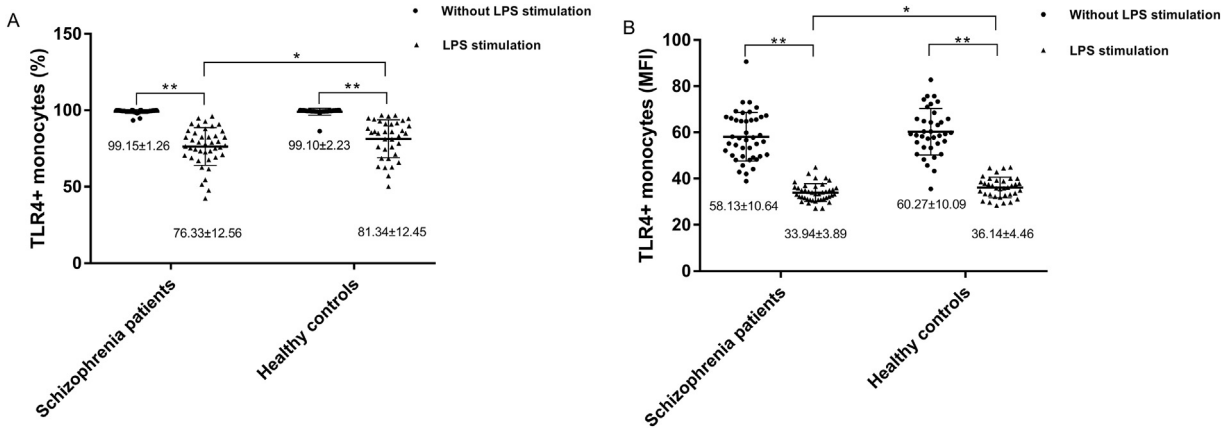

Fig. 2. Comparison of percentage (A) and mean fluorescent intensity (MFI) (B) of monocytes expressing Toll-like receptor 4 (TLR4) following lipopolysaccharide (LPS) stimulation between schizophrenia patients and healthy controls. Corresponding blood samples without LPS stimulation were also incubated in parallel as controls. Error bars represent standard deviation. $^{*}P < 0.05$, $^{**}P < 0.001$.

(Beumer et al., 2012). Therefore, the debilitative effect of impaired TLR4 response to LPS challenge on monocytic function is partial, and its role in the pathophysiology of schizophrenia needs to be addressed further.

It is worth mentioning that our findings showed a down-regulation of TLR4 on monocytes following LPS stimulation compared to unstimulated parallel control samples from all of the participants, and more so in schizophrenia, which is inconsistent to a previous study indicating an up-regulation of TLR4 after stimulation and less increase in schizophrenia than in controls (Muller et al., 2012). One possible interpretation of these results is the different flow cytometric procedures between ours and that study. To detect both cell-surface TLR4 and intracellular cytokines simultaneously, an intracellular protein transport inhibitor (brefeldin A) was added in the LPS-stimulation experiment conducted by Muller et al. (2012), which retained proteins inside cells. However, in our present TLR4 detection, we did not add any protein transport inhibitor, which may underly the discrepancy between these two studies. Moreover, endocytosis of cell surface immune receptors is one of the important dissolutive mechanisms to avoid excessive inflammation caused by immune defense (Plociennikowska et al., 2015). Several studies have demonstrated that LPS induces activated TLR4 complexes to be internalized into endosomes and degraded in lysosomes ultimately, which is an important mechanism of developing endotoxin tolerance (Husebye et al., 2006; Lopez-Collazo and del Fresno, 2013; Nomura et al., 2000; Plociennikowska et al., 2015; Zanoni et al., 2011). In this regard, down-regulation of TLR4 on monocytes following LPS treatment in our present study may be a result of the endotoxin tolerance.

The present study also failed to replicate a report on increased basal expression of monocytic TLR4 in patients with schizophrenia relative to healthy control subjects (Keri et al., 2017a, b; Muller et al., 2012). The main reasons for this inconsistency may be multitude. Firstly, experimental procedures are different. Keri et al. (2017a, b) isolated mononuclear cells by density gradient centrifugation and standard culture medium for immunostaining, whereas we stained the whole blood. Isolated cells lacking their physiological environment may change properties *in vitro*. On the other hand, although the whole blood was also used by Muller et al. (2012), they did not stain the samples immediately after blood drawing as we did, but instead incubated them at 37 °C for 4 h without LPS stimulation as the basal status, which is equivalent to our control blood samples. In our study, we observed a remarkable up-regulation of TLR4 expression on unstimulated control monocytes following 5 h of incubation as compared to the cells stained timely (Supplementary table 1). Furthermore, as discussed earlier, the protein transport inhibitor used in their experiment may also affect TLR4 level on unstimulated monocytes. Secondly, the patients enrolled in the current study had been treated with antipsychotics averagely for 3 days, while the patients from the previous studies had either never received any psychotropic medications or had been free of antipsychotics for at least 4 weeks (Keri et al., 2017a, b; Muller et al., 2012). Several lines of evidence suggest that antipsychotics can dampen TRL4 expression (Keri et al., 2017a; MacDowell et al., 2014). For instance, a single low-dose injection of paliperidone decreased the prefrontal cortical TLR4 expression and suppressed the TRL4-mediated proinflammatory response in rat models (MacDowell et al., 2014). We therefore cannot completely exclude the possibility that short-term antipsychotic treatment may override the otherwise differential expression of TLR4 between patients and controls. Finally, genetic single nucleotide polymorphism (SNP) in TLR4 is known to differ among Asian, African and European populations (Ferwerda et al., 2007). Meanwhile, subjects with the C/C genotype at a TLR4 SNP - rs11536889 expressed a higher level of TLR4 on monocytes than those with the other genotypes (Sato et al., 2012). Thus, interethnic differences in the genetic and epigenetic regulation of TLR4 gene may account for the discrepant results across the different populations. However, since the present and the previous studies (Keri et al., 2017a, b; Muller et al.,

Table 3
Comparison of the MCCB scores between FEDN schizophrenia patients and healthy controls.

Cognition[a]	Schizophrenia (n = 42)	Healthy controls (n = 36)	F Value	p-Value
MCCB composite score	44.08 ± 10.73	60.14 ± 7.99	35.292	1.279×10^{-7}
Processing speed	45.34 ± 11.70	57.96 ± 9.80	13.945	3.836×10^{-4}
Attention/vigilance	40.23 ± 11.20	57.84 ± 7.33	44.903	5.175×10^{-9}
Working memory	44.50 ± 11.71	58.11 ± 6.62	24.190	5.953×10^{-6}
Verbal learning	44.33 ± 12.73	55.35 ± 9.96	10.631	0.002
Visual learning	44.36 ± 10.25	56.32 ± 7.59	21.392	1.697×10^{-5}
Reasoning/problem solving	49.21 ± 11.46	60.17 ± 7.23	8.868	0.004
Social cognition	44.77 ± 11.06	53.76 ± 13.14	8.750	0.004

[a] Analysis of covariance with education years as covariate.

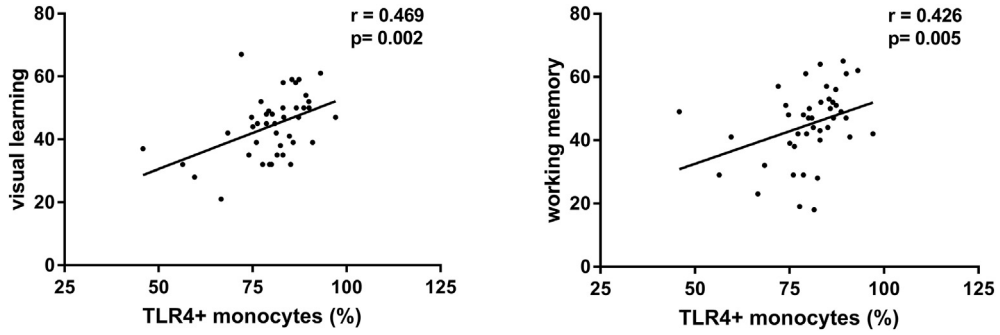

Fig. 3. Correlation analysis revealed a significantly positive association of the basal percentage of TLR4+ monocytes with visual learning ($r = 0.469$, $p = 0.002$) and working memory ($r = 0.426$, $p = 0.005$) in first-episode patients with schizophrenia, which passed Bonferroni test. TLR4 = Toll-like receptor 4.

2012) did not collect genetic information on TLR4 genotypes in the blood samples, our supposition needs a further clarification with a comparatively large sample size.

Our current study found worse neurocognitive functions on the composite MCCB score and all of its subscale scores in FE schizophrenia patients. Since TLR4-mediated low-grade inflammation contributes to cognitive dysfunction in schizophrenia (Aas et al., 2014; Khandaker et al., 2015), we initially expected that TLR4 expression would be associated with cognitive deficits. However, correlation analysis together with multivariate linear regression analysis showed to us that the basal percentage of TLR4 in monocytes was beneficial to visual learning and working memory in FE patients with schizophrenia. This is in disagreement with a recent study showing that higher percentage of TLR4+ monocytes was correlated with more severe cognitive deficits (Keri et al., 2017a). The main reason for this discrepancy is intriguing but might be related to the physiological roles of TLRs in neural plasticity.

TLRs are recently recognized as modulators of CNS development and maturation besides their immune functions (Okun et al., 2011). For instance, TLR4 deficiency increased proliferation and differentiation of neural progenitor cells in the hippocampus, whereas these cells failed to survive and mature into neurons in TLR4-deficient mice (Rolls et al., 2007), suggesting a beneficial effect of TLR4 on neurogenesis and neural plasticity. Another study showed that chronic and mild TLR4 stimulation attenuated Alzheimer's disease-related neuropathology and improved cognitive function in Tau-transgenic mice (Qin et al., 2016). Besides, microglia activated through TLR4 signaling can decrease Aβ deposition and protect from Aβ-mediated neurotoxicity (Song et al., 2011). These studies demonstrate beneficial roles of TLR4 in preventing neuropathology and maintaining cognition in the normal condition, which is corroborative to our current findings. Although no study has ascertained whether TLR4 on circulating monocytes plays a role in neural plasticity, one must not exclude that peripheral innate immune components may influence synapse refinement of the brain (Boulanger, 2009). Given the dual nature and action in a context-dependent manner of TLRs, their putative associations with cognitive performance are sometimes difficult to explain. Therefore, the relationship between TLR4 on monocytes and neurocognition, and the mechanisms underlying their association deserve further exploration.

Several limitations of this study should be noted here. Firstly, intracellular downstream signaling molecules of TLR4 were not detected, such as NF-κB and cytokines levels, although LPS-induced blunted response of monocytic intracellular proinflammatory cytokines in schizophrenia have been demonstrated by other studies (Keri et al., 2017b; Muller et al., 2012). Also, we did not try different concentrations of LPS used for stimulation to explore the monocytic TLR4 response. Hence, these should be confirmed in future studies. Secondly, we did not investigate how antipsychotics treatment may modulate TLR4 expression and activation. Thirdly, due to the cross-sectional nature of the present study design, we currently cannot offer exact explanations for the lower expression of TLR4 on monocytes following LPS stimulation in the patients with schizophrenia compared to healthy subjects. Studies have showed that early life exposure to the bacterial endotoxin reduces the innate immune responsiveness to a subsequent LPS challenge in the adult rat (Ellis et al., 2005; Spencer et al., 2006; Walker et al., 2006), which might be relevant for our finding, particularly since early-life immune activation as a disease primer has been proposed to play an important role in the pathogenesis of neuropsychiatric disorders (Estes and McAllister, 2016). However, we did not acquire the information on infections during early life. Fourthly, due to lacking neuroimaging data, we cannot offer a reasonable neuromechanism underlying the relationships between monocytic TLR4 expression and visual learning and working memory. Finally, the accuracy of these results may be affected by the small sample size; therefore, a replication study should be conducted in a larger cohort and with a longitudinal design to clarify these issues.

In conclusion, this preliminary study showed an exaggerated dampened response of TLR4 to LPS in peripheral blood monocytes of Han Chinese patients with FE schizophrenia, and a protective role of TLR4 for cognitive function, suggesting that TLR4 signaling pathway may be involved in the pathophysiology of schizophrenia. This pathway is related to the innate immune system, further corroborating the role of innate immunity-related functional deficits in schizophrenia susceptibility. Future research is needed to confirm the preventive and therapeutic potential of an appropriate pharmacological modulation of TLR4-dependent pathway in schizophrenia.

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Contributors

Chuan-Yue Wang and Yun-Long Tan designed the experimental protocol, provided the funding for the study and were responsible for the integrity of data and the accuracy of data analysis. Song Chen, Mei-Hong Xiu, Zhi-Ren Wang and Yue-Chan Wang were responsible for recruiting the patients, performing the clinical rating and collecting the samples. Song Chen researched data, analyzed data and wrote the manuscript. Li Tian and Nan Chen were invited in evolving the ideas, analyzing data and editing the manuscript. All authors have contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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