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Antipsychotics influence Toll-like receptor (TLR) expression and its relationship with cognitive functions in schizophrenia

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ABSTRACT

Increasing evidence suggests that altered immune functions are related to the pathophysiology of schizophrenia. Relatively little information is available on Toll-like receptors (TLRs), which are implicated in the recognition of molecular patterns associated with pathogens and internal cellular damage signals. By using immunophenotyping and flow cytometry, we investigated TLRs in CD14+ monocytes, CD4+CD25+Foxp3+ regulatory T cells (T_{reg}), and CD3+CD4+CD25+ activated T cells (T_{act}) in 35 drug-naïve patients with schizophrenia before and after an 8-week period of antipsychotic treatment with risperidone or olanzapine. As compared with 30 healthy control individuals, drug-naïve patients with schizophrenia exhibited an increased percentage of TLR4+ and TLR5+ monocytes and TLR5+ T_{reg}/T_{act} cells. At the end of the treatment period, we observed normalized TLR4+ monocytes and an up-regulation of TLR2+ monocytes and T_{reg}/T_{act} cells. Mean fluorescent intensity values, indicating receptor density, were consistent with these findings. In the drug-naïve state, but not after treatment, higher percentages of TLR4+ and TLR5+ monocytes were correlated with more severe cognitive deficits. Positive, negative, and general clinical symptoms were not associated with TLRs. There were no significant differences between patients receiving olanzapine and risperidone. These results indicate that abnormal expression of TLRs can be detected in the earliest stage of schizophrenia, which is modulated by antipsychotics. Immunological alterations in unmedicated schizophrenia patients may be linked to cognitive deficits.

1. Introduction

The immune hypothesis of schizophrenia is a classic framework for the multifaceted pathophysiology of the illness (DeLisi and Crow, 1986; Ganguli et al., 1994; Girgis et al., 2014; Muller et al., 2000; Sarkar et al., 2010), which has recently been put into the spotlight by the discovery of genetic associations with chromosomal regions spanning major histocompatibility complex (MHC) genes (Stefansson et al., 2009) and complement component 4 (Sekar et al., 2016). Despite extensive research, relatively little information is available regarding a key component of immune regulation, the Toll-like receptors (TLR1-TLR11) in schizophrenia (Venkatasubramanian and Debnath, 2013). These receptors are essential in the first-line recognition of external pathogen-associated molecular patterns (PAMPs) of viruses and bacteria, as well as in the detection of damage-associated molecular patterns (DAMPs), which are cellular components released as a consequence of tissue destruction (Qian and Cao, 2013). When TLRs are activated, they initiate a complex intracellular messenger cascade in immune and glial cells leading to the production of pro-inflammatory cytokines (Akira and Takeda, 2004). From our point of view, it is outstandingly relevant that TLRs are not conservatively included in peripheral immune regulation, but they play a pivotal role in neuroplasticity (neurogenesis, axonal growth, and synaptic remodeling) in the healthy and diseased brain influencing learning, memory, and mood regulation (Garcia Bueno et al., 2016; Hanke and Kielian, 2011; Okun et al., 2011; Trotta et al., 2014), as well as in diverse forms of subtle neuroinflammation and neurodegeneration (Aguirre et al., 2013).

One of the first studies on TLRs and schizophrenia (Chang et al., 2011) reported decreased TLR3 and TLR5 mRNA expression in monocytes, whereas others found increased cytokine production after the stimulation of TLRs (McKernan et al., 2011). Impaired monocyte function was accompanied by higher TLR3 and TLR4

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receptor expression (Muller et al., 2012), and the role of TLR4 was confirmed by postmortem histological studies focusing on the prefrontal cortex (Garcia-Bueno et al., 2016). From a functional point of view, higher TLR expression may be a compensatory phenomenon related to dampened monocyte activation, leading to a perturbed elimination of pathogens and a consequent low-grade inflammation in schizophrenia (Muller et al., 2012). Integrating massive amounts of diverse data, a molecular pathway analysis using genetic loci associated with schizophrenia indicated a key role of molecular pathways linked to the TLRs, which indicates the pivotal role of TLR-related immune mechanisms in the illness (Crisafulli et al., 2015).

In the present study, we aimed at filling some important gaps in the literature. First, it has not been clarified whether TLR expression is altered in drug-naïve, first-episode schizophrenia. Second, it is critical to determine the putative effects of antipsychotics on TLR expression. Third, TLRs should be separately measured in monocytes and T lymphocytes, given that these receptors have distinct functions in different types of immune cells (Oberg et al., 2011; Reynolds and Dong, 2013), and a possible cell-specific disturbance may be important in relation to the exact immune mechanism of schizophrenia. Finally, we were interested in the relationship between clinical/cognitive variables and TLR expression. Taking into consideration previous studies in schizophrenia and other psychotic disorders (Chang et al., 2011; Garcia-Bueno et al., 2016; McKernan et al., 2011; Muller et al., 2012; Wieck et al., 2016) and the technical soundness and reproducibility of measurements, we selected the following targets: TLR1, TLR2, TLR4, TLR5, and TLR6. In T cells, only TLR2 and TLR5 were investigated because in prior pilot and replication studies we could not measure other TLRs properly in these cell types.

We had the following hypotheses: (i) We expected increased TLR expression in schizophrenia relative to healthy control subjects with a special reference to TLR4 (Garcia-Bueno et al., 2016; Muller et al., 2012). (ii) Given that a possible effect of antipsychotics on TLR4 expression was previously suggested (Garcia-Bueno et al., 2016), we hypothesized that an 8-week treatment with antipsychotics will change the expression of TLR4. (iii) Finally, we hypothesized that the severity of clinical symptoms and cognitive deficits would correlate with TLR expression.

2. Materials and methods

2.1. Participants and clinical scales

Thirty-five patients with first-episode schizophrenia and thirty healthy control volunteers matched for age, gender, and education were enrolled (Table 1). The study was carried out at the National Institute of Psychiatry and Addictions, Budapest, Hungary, in collaboration with seven psychiatry centers nationwide. Before the assessment, none of the patients with schizophrenia received psychotropic medications. The mean duration of psychosis with no treatment was 8.0 months (SD = 3.2). The diagnosis was established by using the Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV) (First et al., 1996). We administered the Positive and Negative Syndrome Scale (PANSS) for the evaluation of positive, negative, and general symptoms of schizophrenia (Kay et al., 1987) (Table 1). Individuals with a history of neurological disorders, head injury, inflammatory diseases, current infections, and psychoactive substance misuse (including marijuana) were excluded from the study. Participants were screened for psychoactive substances by using a urine test. The patients and controls did not receive anti-inflammatory medications during a 3-month time interval before the first testing and during the follow-up period. The protocol was reviewed and received ethical approval from the Hungarian Scientific and Research Committee of the Medical Research Council, Budapest, Hungary.

Table 1
Demographic and clinical characteristics of the participants.

	Schizophrenia (n = 35)	Control subjects (n = 30)
Male/female	25/10	21/9
Age (years)	26.7 (7.0)	26.4 (5.5)
Education (years)	11.2 (2.9)	10.7 (2.4)
Body mass index (BMI)	t1: 23.1 (4.0)	t1: 22.4 (4.5)
	t2: 24.2 (3.7)	t2: 22.8 (4.6)
Waist-to-hip ratio	t1: 0.79 (0.13)	t1: 0.78 (0.13)
	t2: 0.83 (0.11)	t2: 0.78 (0.12)
Smokers/non-smokers	16/19	12/18
Positive and Negative Syndrome Scale (PANSS)		
Positive symptoms*		t1: 19.1 (7.8)
		t2: 12.1 (4.5)
Negative symptoms*		t1: 13.7 (6.7)
		t2: 10.3 (6.0)
General symptoms*		t1: 57.1 (16.4)
		t2: 45.6 (12.9)

Data are mean (standard deviation) except for male/female and smokers/non-smokers ratios. There were no significant differences between schizophrenia patients and control subjects as revealed by *t*-tests (for means/standard deviations) and chi-square tests (for distributions) ($p > 0.2$). The PANSS scores showed significant improvement (t1 vs. t2, $p < 0.05$, *t*-tests; for details, see the results section). t1 – baseline testing (unmedicated patients), t2 – follow-up (medicated patients).

2.2. Study design

First, we tested patients with schizophrenia in a drug-naïve state, including clinical evaluation (SCID-CV and PANSS), cognitive assessment, and blood specimen collection. The control subjects also completed the cognitive tests, and blood sample was drawn from them at baseline. In the patient group, the baseline assessment was followed by an 8-week treatment with antipsychotics. The dose of antipsychotic medications was at the discretion of the clinician who was blind to study aims (olanzapine: n = 24, 10–25 mg/day; risperidone: n = 11, 2–6 mg/day). The clinical scales and cognitive tests were administered by trained clinical psychologists and psychiatrist. Following the 8-week treatment period, patients with schizophrenia and control subjects were re-assessed, including clinical scales (patients only), cognitive tests, and blood sample collection.

2.3. Cognitive functions

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) comprises 12 tests from which 5 standardized index scores are calculated (mean: 100, SD = 15, based on data from 200 healthy Hungarian volunteers, 20–80 years of age) (Gold et al., 1999; Juhasz et al., 2003). The RBANS domains are as follows: (1) immediate memory (word list learning, story recall); (2) language (confrontation picture naming, category fluency); (3) visuospatial functions (figure copy, line orientation); (4) attention (digit span, digit-symbol coding); (5) delayed memory (delayed story recall, complex figure, word list recall and recognition). There are two psychometrically matched RBANS forms for repeated testing. In this study, we used the overall RBANS score (average of the five domain scores) to improve statistical power and to avoid multiple comparisons.

2.4. Leukocyte counts

Before flow cytometry measurements, we conducted a routine blood analysis. Leukocyte counts were measured using an LH750