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Plasma cytokines in minimally treated schizophrenia

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ABSTRACT

In schizophrenia, plasma cytokines abnormalities offer vital support for immunopathogenetic basis. However, most of the previous studies on plasma cytokines are confounded by examination of antipsychotic-treated schizophrenia patients. In this study, we examined a large sample of antipsychotic-naïve/free schizophrenia patients ($N = 75$) in comparison with healthy controls ($N = 102$). Plasma cytokines (Interleukins ([IL] 2, 4, 6, 10, 17), Tumor necrosis factor [TNF] and Interferon gamma [IFN-g]) were assessed using cytometric bead array assay. Schizophrenia patients showed significantly greater levels of IL-6 and lower levels of IL-17 as well as IFN-g in comparison to healthy controls. However, after taking censoring into account and adjusting for potential confounders (sex, age, BMI and smoking), only IL-6 was found to be elevated in patients. Cytokine profile showed differential and pathogenetically relevant correlation with clinical symptoms. Together, these observations offer further support to immunological component in schizophrenia pathogenesis.

1. Introduction

In schizophrenia, prenatal maternal immune activation studies (Brown and Derkits, 2010) as well as genetic studies (Hudson and Miller, 2016; Miller and Goldsmith, 2017) strongly suggest an immune basis. Moreover, schizophrenia has been found to be associated with infections, co-existing autoimmune disorders, autoantibodies (Al-Diwani et al., 2017; Severance et al., 2016; Torrey and Yolken, 2017) as well as complement system aberrations (Nimgaonkar et al., 2017). Thus, disparate lines of research strongly suggest that at least a subset of schizophrenia patients are likely to be associated with an immunophenotype (Miller and Goldsmith, 2017).

Among several components of immune pathogenesis, aberrant cytokine signaling is considered as one of the key contributors (Watanabe et al., 2010). Interleukin-6 (IL-6), a pleiotropic cytokine, is reported to be associated with an unfavorable course (Lin et al., 1998), long duration of the illness (Ganguli et al., 1994) and structural brain deficits (Kalmady et al., 2014) in schizophrenia. IL-6 has been shown to influence neuropil pruning in early course schizophrenia (Prasad et al., 2016).

Over the past years, several studies reported elevated levels of IL-6 in schizophrenia (Supplementary Table 1). A recent meta-analysis of blood cytokine network alterations reported a wide ranging array of immune aberrations in schizophrenia (Goldsmith et al., 2016). In this context, it is important to note antipsychotics can alter cytokine levels in schizophrenia (Goldsmith et al., 2016). Since most of the existing studies have examined antipsychotic-treated schizophrenia patients, it has been recommended that further studies should examine untreated patients for cytokine abnormalities (Upthegrove et al., 2014). Hence, in this study, we sought to the evaluate the profile of an array of plasma cytokines and their clinical correlates in a large sample of antipsychotic-naive schizophrenia patients.

2. Material and methods

2.1. Subjects

Schizophrenia patients ($N = 75$; 41-males; age $= 30.69 \pm 6.52$-years; Body Mass Index [BMI] $= 18.97 \pm 3.66$-kg/m^2), who fulfilled DSM-IV criteria [antipsychotic-naïve (never treated with any antipsychotics, $N = 65$) or antipsychotic-free (not exposed to antipsychotics for at least 6 months duration, $N = 10$)] were examined in this study. Mini International Neuropsychiatric Interview (MINI) Plus (Sheehan et al., 1998) was used to ascertain the diagnosis of schizophrenia,

Abbreviations: BMI, Body Mass Index; IFN-g, Interferon gamma; IL, Interleukins; SANS, Scale for Assessment of Negative Symptoms; SAPS, Scale for Assessment of Positive Symptoms; TNF, Tumor necrosis factor.

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which was confirmed by another psychiatrist through an independent clinical interview. Age at onset of psychotic symptoms was 28.08 ± 6.70 years and duration of untreated illness was 2.42 ± 3.12 years. Clinical symptoms were assessed using Scale for Assessment of Positive Symptoms (SAPS) (Andreasen, 1984) (31.29 ± 16.64) and Scale for Assessment of Negative Symptoms (SANS) (Andreasen, 1989) (51.39 ± 25.78).

102 healthy controls (57 males; age = 25.78 ± 4.75-years; BMI = 22.21 ± 2.73-kg/m^2) were recruited from consenting volunteers. Healthy controls were screened using MINI to rule out any psychiatric diagnosis. None of the healthy controls had family history of schizophrenia in any of their first-degree relatives.

None of the study subjects had alcohol abuse/dependence; none used stimulant, opiate, marihuana or cocaine. Proportion of smoking was 29.4% [22 out of 75] in patients and 4.9% [5 out of 102] in controls. None had history suggestive of high grade fever/other features suggestive of infectious/inflammatory disease over the past 6 weeks prior to the assessments. Also, none of the subject had been on treatment with any medications that can potentially affect immune parameters (like steroid). There was no history or clinical features suggestive of neurological/medical diagnosis (including cardiovascular diseases and diabetes mellitus) in any of the study subjects. Subjects were examined with written informed consent with the approval by the institute ethics committee.

2.2. Cytokine assays

Blood samples were collected into K2 EDTA vacutainer tubes (Becton & Dickinson, U.S.A) from all subjects between 0800 and 0900 h. after overnight fast. Plasma was separated by centrifugation, aliquoted and stored at −80 °C. Cytokines (Interleukins [IL]-2, 4, 6, 10, 17 and Tumor necrosis factor [TNF], Interferon gamma [IFN-g]) were assessed using cytometric bead array kit (BD Biosciences, San Jose, USA) (Rao et al., 2015).

2.3. Statistical analysis

Inherent to nature of a typical immunological data, cytokine values were non-normally distributed and consisted of non-detects. For a given cytokine, concentrations below its reporting threshold were considered non-detectable and hence left-censored. Statistical analyses were done using R programming language (R Core Team, 2013) with RStudio integrated development environment version 0.98 (RStudio, 2013).

Tobit regression on ranks of cytokine values, a recommended statistical approach in non-normal immunological data with non-detects (Ballenberger et al., 2012), was performed for each cytokine separately. This method allowed for co-varying variables such as sex, age, BMI and smoking status to control for their potential confounding effect on the cytokine values. Summary statistics and regression equations for singly censored data were computed using Maximum Likelihood Estimation (Helsel, 2005; Meeker and Escobar, 2014). The Mann–Whitney *U* test as well as censored regression without and with co-varying for potential confounders were performed to test the differences in cytokine levels between the controls and patients using R package NADA version 1.5-6. (Lee, 2013) (http://CRAN.R-project.org/package=NADA).

A data reduction approach was used to visualize each subject's cytokine data in a 2-dimensional space to identify differences in cytokine patterns between the study groups. Multiple factor analysis was done to study association of Th1 [IFN-g,TNF, IL-2], Th2 [IL-10, IL-4], Th17 [IL-17] and regulatory cytokine IL-6 related immune response with the disorder and its clinical characteristics, while balancing the influence of each group of cytokines. Diagnostic status of subjects [schizophrenia patient or healthy control] and variables other than cytokines were treated agnostically as supplementary and hence did not influence the eigenvectors/eigenvalues or loadings but are projected on the results drawn from active variables. In order to accommodate for censored data, cytokine values were rank transformed (Helsel, 2011). Number of dimensions was selected based on "leveling off" of Eigen values on the screen plot. The procedure and graphical representations were implemented using R package FactoMineR version 1.27 (Husson et al., 2014) (http://CRAN.R-project.org/package=FactoMineR), 'ggplot2' version 1.0.1 (Wickham, 2009), 'gridExtra' version 0.9.1 (Auguie, 2012).

3. Results

Patients and controls did not differ significantly in sex ratio (χ^2 = 0.03; p = 0.87); age (t = 5.52; $p < 0.001$) and BMI (t = 6.44; $p < 0.001$) were significantly different. Based on the lowest reported concentrations, we set the reporting limit of cytokines IL-17, IFN-g, TNF, IL-10, IL-6, IL-4, IL-2 to be 3.02, 1.21, 1.00, 1.01, 1.02, 1.10, 1.10 pg/mL respectively for our assays. Concentrations below these thresholds were considered as non-detects and hence, were left-censored. Overall, percentage of censored values for IL-17, IFN-g, TNF, IL-10, IL-6, IL-4, IL-2 were 75%, 76%, 67%, 49%, 14%, 72%, 89% respectively.

Differences in cytokine levels between patients and controls are described in Table 1 and Supplementary Fig. 1. Results revealed significantly greater levels of IL-6 & lower levels of IL-17 as well as IFN-g in schizophrenia patients in comparison to healthy controls. However, after taking censoring into account and adjusting for potential confounders (age, sex, BMI and smoking) only IL-6 was found to be significantly greater in patients. Analyses on antipsychotic-naïve patients alone (excluding antipsychotic-free ones) showed that group differences in IL-6 levels persisted to be significant (Supplementary Table 2).

Multiple factor analysis was applied to test the polarization of Th related responses. Multiple factor analysis of ranked cytokine data for

Table 1
Comparative profile of plasma cytokine levels between schizophrenia patients and healthy controls.

Cytokine	Plasma cytokine levels [pg/ml]		U[a]	Z[a]	p[a]	Z[b]	p[b]	Z[c]	p[c]
	MLE Mean ± SD								
	Patient (n = 75)	Control (n = 102)							
IL-17a	9.96 ± 311.82	11.58 ± 62	3046	2.84	0.005	2.96	0.003	1.25	0.21
IFN-g	1.22 ± 6.56	1.38 ± 1.58	3217	2.27	0.023	2.45	0.01	1.36	0.17
TNF	1 ± 1.06	1.07 ± 0.88	3633	0.67	0.502	0.64	0.49	0.61	0.54
IL-10	1.83 ± 3.4	1.59 ± 1.07	3291	1.67	0.096	1.79	0.07	0.14	0.88
IL-6	3.47 ± 3.07	2.33 ± 1.33	2835	2.94	0.003	2.93	0.003	2.15	0.03
IL-4	1.2 ± 2.19	1.11 ± 1.35	3801	0.09	0.93	0.1	0.91	0.43	0.66
IL-2	0.5 ± 2.18	0.69 ± 1.09	3588	1.2	0.229	1.23	0.22	0.67	0.51

MLE - Maximum likelihood estimate.
[a] Mann–Whitney *U* test.
[b] Tobit regression on ranks taking censoring into account.
[c] Tobit regression on ranks taking censoring into account and adjusting for potential confounders - sex, age, BMI and smoking.

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