OXFORD

International Journal of Neuropsychopharmacology, 2015, 1–8

doi:10.1093/ijnp/pyu042
Research Article

RESEARCH ARTICLE

Effects of Risperidone on Cytokine Profile in Drug-Naïve First-Episode Psychosis

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Abstract

Background: There is robust evidence that schizophrenia is characterized by immune-inflammatory abnormalities, including variations on cytokine levels. The results of previous studies, however, are heterogeneous due to several confounding factors, such as the effects of antipsychotic drugs. Therefore, research on drug-naïve first-episode psychosis (FEP) patients is essential to elucidate the role of immune processes in that disorder.

Methods: The aim of this study is to compare cytokine levels (IL-2, IL-10, IL-4, IL-6, IFN-γ, TNF-α, and IL-17) in drug-naïve FEP patients both before and after treatment with risperidone for 10 weeks, and to investigate possible associations between cytokine levels and clinical responses to treatment and presence of depressive symptoms. It this study, we included 55 drug-naïve FEP patients who had repeated measurements of cytokine levels and 57 healthy controls.

Results: We found that FEP patients had significantly higher IL-6, IL-10 and TNF-α levels than healthy controls. After risperidone treatment, these three cytokines and additionally IL-4 decreased significantly. No significant difference was found between the post-treatment cytokine levels in FEP patients and in healthy controls, suggesting that these alterations in cytokine profiles are a state marker of FEP. No significant association was found between risperidone-induced changes in cytokines and the clinical response to treatment or the presence of depression. There was a significant inverse association between the risperidone-induced changes in IL-10 and the negative symptoms.

Received: August 4, 2014; **Revised:** September 5, 2014; **Accepted:** September 9, 2014

Conclusions: In conclusion, our results show a specific cytokine profile in FEP patients (monocytic and regulatory T-cell activation) and suggest immunoregulatory effects of risperidone treatment, characterized by suppressant effects on monocytic, Th2, and T-regulatory functions.

Keywords: antipsychotic, cytokines, drug-naïve, FEP, immunology, inflammation, risperidone, schizophrenia

Introduction

Abnormalities in immune-inflammatory pathways have consistently been described in schizophrenia (Smith and Maes, 1995; Anderson et al., 2013). Increased levels of pro-inflammatory cytokines and T helper (Th) 1-like cytokines were reported in schizophrenia, suggesting, respectively, macrophage and T-cell activation (Miller et al., 2011). A recent meta-analysis showed an elevation in pro-inflammatory cytokine (and receptor) levels in the serum of drug-naïve first-episode psychosis (FEP) patients with higher levels of interleukin (IL)-1β, soluble IL-2 receptor (sIL-2R), IL-6, and tumor necrosis factor (TNF)-α, a profile indicative of M1 (macrophage1) and Th1 activation (Upthegrove et al., 2014). These changes in cytokine profile accompany the onset of the disorder (Borovcanin et al., 2012; Di Nicola et al., 2013).

Antipsychotics have a variety of effects on cytokine levels (Maes et al., 1994, 2000; Zajkowska and Mondelli, 2014). Tourjman et al. (2013), in a meta-analysis, found increased levels of sIL-2R and reduced levels of IL-1β and interferon (IFN)-γ after antipsychotic treatment in individuals with schizophrenia. However, it has remained elusive whether these changes in cytokine levels are due to effects of the antipsychotic drugs per se, or whether they are a consequence of psychopathology improvement. Even using the same antipsychotic agent (e.g., risperidone), results remain heterogeneous, with differences between *in vitro* and *in vivo* studies, stages of illness, and schizophrenia subtypes. *In vitro*, Himmerich et al. (2011) showed that several antipsychotics increase IL-17 levels and that risperidone may inhibit Th1 cytokines (e.g., IL-12) and increase Th2 cytokines (e.g., IL-10) and pro-inflammatory cytokines, such as IL-6, IL-8, and TNF-α (Chen et al., 2012). On the other hand, Kato et al. (2007) found that risperidone significantly inhibits the production of IL-1β, IL-6, and TNF-α by IFN-γ-activated microglia. In an animal model of neuroinflammation using a lipopolysaccharide (LPS) challenge, atypical antipsychotics, including risperidone, suppress TNF-α and IL-6, and up-regulate IL-10 (Sugino et al., 2009). Interestingly, risperidone also prevented the increase of inflammatory parameters induced by LPS and restored anti-inflammatory pathways decreased by LPS (MacDowell et al., 2013). *In vivo*, typical antipsychotics suppressed plasma IL-6 and soluble IL-6 receptors (Maes et al., 1995), whereas the administration of clozapine and risperidone increased sIL-2 receptor, IL-6, and TNF-α (Maes et al., 1994, 1997). More recently, it was shown that treatment with atypical antipsychotics may increase type-2 cytokine serum levels in schizophrenia, and decreases IL-4, IL-6, and IL-27 levels (Borovcanin et al., 2013). Many confounding factors may result in inconsistent results among studies, including gender, smoking habits, age, stages of illness, general medical comorbidities, and differences between antipsychotic agents (Haack et al., 1999). Furthermore, it has been demonstrated that subgroups of schizophrenia (e.g., depressed or treatment-resistant) may present distinct cytokine profiles (Noto et al., 2011, 2013).

The aim of this study was to delineate the effects of risperidone on the levels of M1 (IL-6, TNF-α), Th1 (IL-2, IFN-γ), Th2 (IL-4), and T-regulatory cell (IL-10) cytokines in a sample of drug-naïve FEP patients and to delineate whether the effects of risperidone on cytokine levels are related to a good clinical response or to changes in depressive symptoms in FEP patients.

Subjects and Methods

This study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Research Ethics Committee of the Universidade Federal de São Paulo, and all participants provided written informed consent prior to enrollment.

Subjects

We recruited antipsychotic-naïve FEP patients (n = 55) admitted to a psychiatric emergency unit in São Paulo, Brazil. For the purpose of the study, FEP was defined by a distinct period characterized by the emergence of psychotic symptoms. To delineate the beginning of the episode, we investigated the last period before the first onset of psychotic symptoms, including familiar interviews when necessary. All patients fulfilled one of the following diagnoses, according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, using their Structured Clinical Interview (SCID-I): schizophrenia, schizophreniform disorder, brief psychotic disorder, and psychotic disorders not otherwise specified. The mean time between the onset of psychotic symptoms and the introduction of treatment was 223 days (±435). A history of cannabis use was reported by 27.3% of the patients. Nevertheless, none of them fulfilled criteria for a substance-induced psychotic disorder. The sample included individuals between 16 and 40 years old, who had never used any antipsychotic before admission. Exclusion psychiatric criteria were: psychotic episodes due to a general medical condition, substance-induced psychotic disorder, intellectual disability, and a psychotic episode associated with bipolar or major depressive disorders. General exclusion criteria were: acute and chronic general medical conditions associated with abnormalities in immune-inflammatory responses, such as infections, HIV, allergies, pregnancy or the postpartum period; rheumatologic or immunological conditions were also exclusionary criteria. In addition, individuals who had used medications with immunomodulatory effects such as non-steroidal anti-inflammatory drugs, corticosteroids, and immunosuppressant drugs were excluded.

All patients received risperidone as standard treatment. The mean dose of risperidone was 4.0 mg (±1.8). The titration was performed according to clinical judgment. Other medications were used in the period between both assessments, according to the symptomatology. The drugs used were benzodiazepine (16.4%), anticholinergic (9%), and an antidepressant (3.6%).

The comparison group consisted of antipsychotic-naïve, healthy volunteers (n = 57) assessed for personal and familiar history of psychiatric disorders according to the criteria of the SCID-I. Only individuals without a current or lifetime history of psychiatric disorders and with an absence of a major mental disorder in first-degree relatives were included. The general exclusion criteria were the same as with the individuals with FEP.

The relative severity of illness dimensions was assessed using: (a) the Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987); (b) the Clinical Global Impression Scale (CGI; Guy et al., 1976); (c) the Global Assessment of Functioning Scale (GAF; American Psychiatric Association, 2000); and (d) the Calgary Depression Scale for Schizophrenia (CDS; Addington et al., 1990).

Response to treatment was defined as a reduction of more than 50% of baseline PANSS total score (Leucht et al., 2009). The percentage reduction in the total PANSS score from baseline to endpoint was calculated after subtracting the 30 minimum points of the total PANSS score (Leucht et al., 2009). We divided the patient group into those with and without depression using a threshold value of CDS > 5. According to the Brazilian version of CDS, this cutoff value presents a specificity of 92% and a sensitivity of 77% to identify major depression (Bressan et al., 1998).

Methods

Blood samples of 10mL were withdrawn from all patients at admission, before the first dose of risperidone, and after a mean of 10.95 weeks of treatment (standard deviation 5.7 weeks), and from all healthy controls after the SCID-I interview. Blood was immediately centrifuged and the serum was stored at –80ºC until thawed for assays of cytokines. All samples were thawed the same day and assays were carried out in a single run using the same batch of reagents. Multiple cytokines (IL-2, IL-10, IL-4, IL-6, IFN-γ, TNF-α, and IL-17) were measured by flow cytometry using the Cytometric Bead Array Human Th1/Th2/Th17 Kit (BD Biosciences). Acquisition was performed with a FACS Canto II flow cytometer (BD Biosciences). The instrument was checked for sensitivity and overall performance with Cytometer Setup and Tracking beads (BD Biosciences) prior to data acquisition. Quantitative results were generated using FCAP Array v1.0.1 software (Soft Flow Inc.). IL-2 and IFN-γ were measurable only in a few patients and controls and therefore analyses could not be carried out.

Statistical Analyses

Statistical analyses levels were performed using SPSS v.20. We used analyses of variance (ANOVAs) and analyses of contingency tables (χ^2 tests) to examine differences in socio-demographic and clinical data between the study samples. Univariate and multivariate general linear model (GLM) analyses were used to examine the effects of explanatory variables (diagnostic groups and socio-demographic data) on the cytokine levels. Repeated measurements (RM) design ANOVAs were used to examine the effects of treatment (time) and time x diagnostic group interactions as within-subject contrasts. Relationships between variables were assessed by means of regression analyses and Pearson's product moment correlation analyses. We also used Spearman correlation calculations to check the results of parametric methods. Statistical significance was set at alpha ≤ 0.05 (two-tailed).

Results

Characteristics of the Sample

Table 1 describes the socio-demographic and clinical data of FEP patients and healthy controls. No significant differences were found between the two groups in either age or gender. Without Bonferroni *p* correction for multiple statistical analyses and comparisons, there were significant differences in years of education and income between psychotic patients and controls. Table 1 also shows the mean values of the rating scales in the FEP patients.

Effect of Risperidone Treatment on Cytokines and Clinical Levels

Table 2 shows the cytokine level measurements at baseline and post-treatment in FEP patients when compared to healthy controls. Table 2 also shows the comparison between pre- and post-treatment cytokines and psychopathology data as a time factor and a time x gender interaction, analyzed with RM design ANOVAs. Treatment with risperidone significantly lowered IL-6, IL-4, IL-10, and TNF-α, while there was a trend towards increased IL-17 levels after treatment. There were no significant interactions between time x gender. Treatment with risperidone reduced the PANSS general, PANSS positive, CDS, CGI, and GAF scores. There was no significant effect of treatment with risperidone on the PANSS negative subscale score. Changes in the levels of cytokines were not correlated with the dosage of risperidone ($p < 0.05$).

Table 1. Socio-demographic and Clinical Characteristics of First-episode Psychosis (FEP) Patients and Healthy Controls (HC).

	FEP n = 55	HC n = 57	F or X^2	df	p
Gender			1.90	1	0.168
Male	65.5%	52.6%			
Female	34.5%	47.4%			
Age	24.75 (±7.43)	26.61 (±7.53)	-1.32	1/110	0.189
Marital status			4.30	1/2	0.117
Single	87.5%	78.8%			
Marriage	7.5%	21.2%			
Divorced	7.5%	0%			
Years of education			15.15	1/1	<0.001
<11 years	47.5%	6.1%			
≥11 years	52.5%	93.9%			
Income (US dollars/per month)	363.85 (±262.67)	1148.65 (±857.43)	5.40	1/67	<0.001
Smoking			7.14	1/1	0.008
Yes	18.9%	0%			
No	81.1%	100%			
PANSS positive	28.27 (±5.53)	-			
PANSS negative	21.88 (±6.89)				
PANSS general	49.12 (±13.12)				
PANSS Total	99.27 (±20.64)				
CDS	5.37 (±6.00)	-			
CGI	5.13 (±0.74)	-			
GAF	27.26 (±10.08)	-			

CDS: Calgary Depression Scale; CGI: Clinical Global Impression; GAF: Global Assessment of Functioning; PANSS: Positive and Negative Syndrome Scale, Results are shown as mean ± standard deviation.

Results of ANOVAs showed that pre-treatment IL-6 levels in FEP patients were significantly higher than in controls (F = 9.89, df = 1/112, p = 0.002), whereas post-treatment IL-6 values in FEP patients were not significantly different from those in controls (F = 0.41, df = 1/110, p = 0.524). Pre-treatment IL-4 levels of FEP patients were not significantly different from IL-4 levels in controls (F = 2.15, df = 1/110, p = 0.145), whereas the post-treatment IL-4 levels were significantly lower in FEP patients than in controls (F = 6.00, df = 1/110, p = 0.016). The pre-treatment IL-10 levels of FEP patients were significantly higher than those in healthy controls (F = 18.82, df = 1/110, p < 0.001), whereas the post-treatment IL-10 values in FEP patients were not significantly different from those in controls (F = 0.01, df = 1/110, p = 0.939). The baseline TNF-α values in FEP patients were significantly higher than those in healthy controls (F = 8.87, df = 1/110, p = 0.004), whereas the post-treatment TNF-α values in FEP patients were not significantly different from those in controls (F = 3.90, df = 1/110, p = 0.051). There were no significant differences between the IL-17 values in the controls and the baseline (F = 1.27, df = 1/110, p = 0.260) or post-treatment IL-17 (F = 0.91, df = 1/110, p = 0.343) values in FEP patients. Multivariate GLM analysis showed that the pre-treatment levels of the five cytokines (IL-4, IL-6, IL-10, TNF-α, and IL-17) in the FEP patients were significantly different from controls (F = 5.74, df = 5/106, p < 0.001), while there were no significant differences in the post-treatment levels of the five cytokines in the FEP patients and the controls (F = 2.13, df = 5/106, p = 0.067). We examined the effects of current tobacco use disorders on the cytokine levels and a possible interaction with the effects of risperidone. Multivariate GLM analysis did not show any significant effect of current tobacco use disorder on the cytokine levels (F = 0.92, df = 5/64, p = 0.472). We were unable to find any significant time x current tobacco use disorder and diagnostic group x time x current tobacco use disorder for any of the five cytokine levels.

Responder and Non-Responders Analysis on Clinical Parameters

Table 3 shows the results of RM design ANOVAs with time (pre- and post-treatment PANSS and CDS scores) and time x responder status (responder versus non-responder status based on changes in the PANSS total score) as within-subject contrasts. RM design ANOVAs showed significant interactions between time x responder status for the total PANSS as well as the PANSS positive, negative, and general psychopathology subscales. Treatment with risperidone showed a significant suppressant effect on the CDS score, although there were no significant differences between responders and non-responders to treatment.

Table 2. Effects of Treatment with Risperidone on Serum Cytokine Levels in First-episode Psychosis (FEP) Patients. The Pre- and Post-treatment Data in the FEP Patients Are Compared with Those in Healthy Controls.

	HC (n = 57)	FEP Pre (n = 55)	FEP Post (n = 55)	Time F-value*	df	p	Time x Gender F-value*	p
IL-6	0.77 (±0.04)	1.64 (±1.77)	0.90 (±0.81)	9.81	1/53	0.003	0.11	0.745
IL-4	0.24 (±0.63)	0.43 (±0.78)	0.03 (±0.09)	16.21	1/53	<0.001	0.14	0.291
IL-10	0.20 (±0.45)	0.84 (±1.01)	0.21 (±0.34)	18.71	1/53	<0.001	0.57	0.453
TNF-α	0.16 (±0.51)	0.63 (±1.07)	0.02 (±0.02)	17.69	1/53	<0.001	0.00	0.985
IL-17	4.26 (±11.87)	2.31 (±4.76)	6.70 (±15.11)	3.11	1/53	0.083	0.49	0.489
PANSS Pos	-	28.38 (±5.62)	13.54 (±5.17)	118.65	1/35	<0.001	0.84	0.773
PANSS Neg	-	22.22 (±7.02)	20.51 (±7.48)	0.41	1/35	0.435	0.41	0.525
PANSS Gen	-	50.05 (±13.33)	33.51 (±8.25)	47.70	1/35	<0.001	1.51	0.227
PANSS Total	-	100.65 (±21.14)	60.4 (±17.66)	60.45	1/35	<0.001	0.35	0.557
CDS	-	5.60 (±6.23)	2.83 (±4.37)	7.99	1/33	0.008	2.42	0.129
CGI	-	5.18 (±0.73)	3.33 (±1.31)	40.24	1/31	<0.001	0.77	0.388
GAF	-	27.00 (±9.56)	57.12 (±16.63)	76.83	1/31	<0.001	1.88	0.180

CDS: Calgary Depression Scale; CGI: Clinical Global Impression; GAF: Global Assessment of Functioning; IL: interleukin; PANSS: Positive and Negative Syndrome Scale; TNF: tumor necrosis factor.
Results are shown as mean ± standard deviation and are in pg/mL.*Results of repeated measurements design ANOVAs are with risperidone treatment as time factor and gender as between-subject factor (and performed on FEP Pre versus FEP Post).

Table 3. Effects of Treatment with Risperidone on Psychopathology in First-episode Psychosis (FEP) Patients, Comparing the Pre- and Post-treatment Data in FEP Patients Who Were Responders or Non-responders to Treatment with Risperidone.

		Responders	Non-responders	Time F-value*	df	p	Time x Responder status F-value*	df	P
CDS	PRE	6.56 (±5.89)	4.79 (±6.54)	5.91	1/35	0.020	1.66	1/35	0.207
	POST	2.13 (±3.30)	3.42 (±5.11)						
PANSS Total	PRE	104.39 (±21.60)	97.10 (±20.64)	163.76	1/35	<0.001	39.41	1/35	<0.001
	POST	54.33 (±8.34)	80.00 (±14.82)						
PANSS Pos	PRE	29.89 (±4.78)	26.95 (±6.09)	255.65	1/35	<0.001	21.36	1/35	<0.001
	POST	10.61 (±2.52)	16.32 (±5.54)						
PANSS Neg	PRE	22.44 (±8.06)	22.00 (±6.10)	2.99	1/35	0.09	19.08	1/35	<0.001
	POST	16.00 (±4.70)	24.79 (±7.17)						
PANSS Gen	PRE	52.06 (±13.58)	48.16 (±13.16)	75.87	1/35	<0.001	15.58	1/35	<0.001
	POST	27.72 (±4.08)	39.00 (±7.42)						

CDS: Calgary Depression Scale; PANSS: Positive and Negative Syndrome Scale.
*Results of repeated measurements design ANOVAs with risperidone treatment as the time factor and gender as the inter-individual factor. Results are shown as mean ± standard deviation.

Table 4 shows the results of RM design ANOVAs with time (pre- and post-treatment cytokine levels) and time x responder status (responders versus non-responder status to treatment) as within-subject contrasts. There were no significant interactions between time x treatment responder status for any of the cytokines.

Correlation Between Cytokines Levels and the Presence of Depression

Table 5 shows the results of RM design ANOVAs with time (pre- and post-treatment cytokines data) and time x diagnostic depression groups (patients divided according to the baseline CDS values using a threshold value >5) as within-subject contrasts. We found significant interactions between time x diagnostic groups for IL-4 and TNF-α. Thus, treatment with risperidone significantly decreased IL-4 and TNF-α in FEP patients with depressive symptoms and not in those without depressive symptoms.

Correlation Between Cytokines Levels and Psychopathology

We also examined the correlations between the changes in cytokines and psychopathology levels from baseline to endpoint. The changes from baseline to endpoint were computed as the residual values obtained by the regression of the endpoint values on the baseline values (and secondly by computing the delta values: that is, endpoint minus the baseline values). There were significant correlations between the residual changes in the PANSS positive and PANSS negative subscale scores (r = 0.336, p = 0.042). There were no significant correlations between the residual changes in the CDS score and those in the PANSS total or PANSS positive or negative subscales. There was a significant inverse association between the residual changes in IL-10 and those in the PANSS negative score (r = -0.480, p = 0.001). There were no significant associations between the residual changes in the CDS scores and any of the cytokines measured.

In order to examine possible effects of age on the effects of risperidone, we carried out RM ANOVAs with age groups as an additional factor. The study sample was divided into two age groups: subjects aged <20 years old (n = 23) and subjects ≥20 years old (n = 32). We did not detect significant interactions between time and age groups on IL-6 (F = 3.91, df = 1/53, p = 0.053), IL-4 (F = 1.23, df = 1/53, p = 0.272), IL-10 (F = 0.24, df = 1/53, p = 0.628), TNFα (F = 1.45, df = 1/53, p = 0.234), or IL-17 (F = 0.82, df = 1/53, p = 0.370).

Discussion

The first major finding of this study is that treatment with risperidone for 10 weeks had a significant suppressant effect

Table 4. Effects of Treatment with Risperidone on Serum Cytokine Levels in First-episode Psychosis (FEP) Patients, Comparing the Pre- and Posttreatment Data in the FEP Patients Between Treatment Responders and Non-responders.

		Responders	Non Responders	Time F-value*	df	p	Time x Responder status F-value*	df	p
IL-6	PRE	1.92 (±2.01)	1.49 (±1.95)	6.21	1/35	0.018	0.00	1/35	0.950
	POST	1.09 (±1.09)	0.70 (±0.44)						
IL-4	PRE	0.48 (±0.18)	0.34 (±0.78)	8.73	1/35	0.006	0.17	1/35	0.681
	POST	0.05 (±0.14)	0.02 (±0.05)						
IL-10	PRE	1.13 (±1.13)	0.75 (±0.89)	13.54	1/35	<0.001	0.14	1/35	0.715
	POST	0.38 (±0.47)	0.13 (±0.26)						
TNF-α	PRE	0.80 (±1.46)	0.42 (±0.71)	11.23	1/35	0.002	0.98	1/35	0.330
	POST	0.05 (±0.17)	0.10 (±0.00)						
IL-17	PRE	2.41 (±4.22)	3.81 (±6.52)	2.57	1/35	0.118	0.80	1/35	0.378
	POST	9.99 (±21.93)	5.97 (±13.17)						

IL: interleukin; TNF: tumor necrosis factor.
*Results of repeated measurements design ANOVAs with risperidone treatment as the time factor and responder status as the inter-individual factor. Cytokine levels are shown as mean ± standard deviation and are in pg/mL.

Table 5. Effects of Treatment with Risperidone on Serum Cytokine Levels in First-episode Psychosis (FEP) Patients, Comparing the Pre- and Posttreatment Data in the FEP Patients with (CDS > 5) and Without (CDS ≤ 5) Depression as Measured by Means of the Calgary Depression Scale Score.

		CDS ≤ 5	CDS > 5	F-value*	df	p	F-value*	df	p
IL-6	PRE	1.46(±1.87)	1.75 (±1.78)	5.13	1/34	0.030	0.19	1/34	0.668
	POST	0.89 (±1.09)	0.91 (±1.22)						
IL-4	PRE	0.17 (±0.48)	0.85 (±1.01)	14.77	1/34	<0.001	6.19	1/34	0.013
	POST	0.20 (±0.05)	0.06 (±0.16)						
IL-10	PRE	0.98 (±1.13)	0.92 (±0.84)	14.48	1/34	<0.001	0.07	1/34	0.800
	POST	0.29 (±0.44)	0.14 (±0.23)						
TNF-α	PRE	0.27 (±0.58)	1.24 (±1.61)	18.00	1/34	<0.001	7.37	1/34	0.010
	POST	0.01 (±0.00)	0.07 (±0.21)						
IL-17	PRE	1.93 (±0.35)	5.49 (±7.60)	1.46	1/34	0.235	0.48	1/34	0.495
	POST	8.12 (±19.76)	7.18 (±15.33)						

CDS: Calgary Depression Scale; IL: interleukin; TNF: tumor necrosis factor.
Cytokine levels are shown as mean ± standard deviation and are in pg/mL. *Results of repeated measurements design ANOVAs with risperidone treatment as the time factor and depression as the inter-individual factor.

on several serum cytokine levels. The baseline levels of IL-6, IL-10, and TNF-α were significantly higher in drug-naïve FEP patients when compared to healthy controls but were normalized after 10 weeks of risperidone treatment, whereas IL-4 levels reduced. These results suggest a specific cytokine profile in FEP patients characterized by monocytic and T-regulatory cell responses, and indicate that treatment with risperidone may normalize these responses and additionally decrease Th2 functions. Based on our results, we conclude that risperidone seems to normalize an aberrant cytokine profile in FEP. Therefore, the immune effects of risperidone in FEP cannot be described as either anti-inflammatory or "negative immunoregulatory".

Our results are partially in agreement with previous papers reporting on the effects of risperidone and other antipsychotic agents. In a drug-naïve FEP sample, Song et al. (2014) found increased levels of baseline IL-1β, IL-6, and TNF-α in FEP patients compared to controls. Following risperidone treatment, they found an increase in TNF-α and an initial decrease of IL-6 levels succeeded by an increase to baseline levels. Risperidone attenuates the increase of inflammatory parameters induced by LPS and restores anti-inflammatory pathways decreased by LPS (MacDowell et al., 2013). Other studies found different cytokine responses to risperidone, likely attributable to differences in media (immune cells versus microglia), *in vitro* and *in vivo* studies, and staging of illness and schizophrenia subtypes (Kato et al., 2007; Sugino et al., 2009; Himmerich et al., 2011; Chen et al., 2012; Borovcanin et al., 2013; MacDowell et al., 2013; Song et al., 2014).

Significant immune effects of other antipsychotic drugs in FEP patients have been reported (see Tourjman et al., 2013). These previous studies found a significant decrease of IL-6, IL-4, and IL-27 after antipsychotic treatment in FEP (Kubistova et al., 2012; Borovcanin et al., 2013), but not of TNF-α (Kubistova et al., 2012). In a recent meta-analysis (Tourjman et al., 2013), 23 studies were included showing that antipsychotics may have anti-inflammatory effects. However, none of the cytokine levels reduced after risperidone treatment in our study were significant in this meta-analysis. These differences may be explained by differences in study samples and antipsychotic agents, such as FEP patients treated with risperidone in our study versus chronic patients treated with different antipsychotics in the meta-analysis. In another meta-analysis, Miller et al. (2011) concluded that some cytokines (IL-1β, IL-6, and TGF-β) may be state markers for the acute phase of schizophrenia, while other immune markers (IL-12, IFN-β, TNF-β, and sIL-2R) may be trait markers. However, in our sample, we did not find any cytokine that could function as a trait marker because all cytokines that were initially increased in FEP were normalized after risperidone treatment.

The second major finding of this study is that risperidone treatment improved psychopathology, and that this effect was not related to risperidone-induced changes in cytokine levels from baseline to endpoint. Interestingly, risperidone treatment had a significant effect on the severity of positive and depressive symptoms and general pathology as measured with the CGI or GAF, but had no significant effect on the negative symptoms. We were unable to establish a significant association between a good clinical response to treatment (as defined by a 50% reduction in the PANSS total scale) and the risperidone-induced changes in cytokine levels. Therefore, we conclude that the normalization of the cytokine profile by risperidone treatment should be ascribed to the actions of risperidone, and that it occurs independently from changes in psychopathological improvement. Interestingly, we could not find a correlation between the doses of risperidone and the post-treatment cytokine levels or risperidone-induced cytokine changes.

Another important question is whether there are significant differences in the effects of risperidone between individuals with and without depression (as measured with the CDS score). We found that treatment with risperidone significantly decreased IL-4 and TNF-α in FEP patients with depressive symptoms, but not in those without depressive symptoms. These findings show that risperidone may have different immune effects in FEP patients with depression as compared to FEP patients without depression.

In regard to the effect of risperidone on psychopathology, we found that while there was no significant overall effect of risperidone treatment on the PANSS negative symptom score, there was a significant time x responder treatment interaction. Thus, after treatment with risperidone the negative symptom severity was increased compared to baseline, whereas in responders negative symptoms were decreased. This suggests that non-reactivity in negative symptoms is strongly associated with non-responder status. Interestingly, the risperidone-induced changes in the negative symptoms were strongly and inversely correlated with those in IL-10. Thus, it appears that a comparatively smaller suppressant effect of risperidone on IL-10 may be protective against negative symptoms. This is important, since IL-10 is an anti-inflammatory and immunoregulatory cytokine that has neuroprotective effects (Arimoto et al., 2007). Such an effect may be related to a better clinical outcome.

The longitudinal assessment of drug-naïve FEP patients with the standardization of the antipsychotic treatment is the main strength of our study and adds to the current literature on the immunomodulation of antipsychotics, specifically risperidone. Therefore, this study was controlled for two very important confounding factors. However, some limitations should be mentioned. IL-2 and IFN-γ serum levels were measurable only in a few patients and controls and therefore analyses could not be carried out. Furthermore, the analysis was made after 10 weeks, and it would have been more informative to have data also at later time points. A longer follow-up period could be helpful to clarify the long-term effects of risperidone.

In summary, we found that risperidone significantly normalized the initially-abnormal cytokine profiles in FEP patients. This normalization of the cytokine profiles was, however, not associated with a good clinical response to risperidone. This suggests that the normalization of the cytokine profiles was due to specific effects of risperidone and was not secondary to symptomatic improvement. The inverse correlation between risperidone-induced changes in IL-10 and negative symptoms indicates that IL-10 may have protective effects against negative symptoms.

Acknowledgments

The authors are thankful to Prof. Alexandre Basso and to Leandro Pires Araújo for their valuable help in measurement of cytokines.

This study was funded through the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2010/08968-6, 2010/19176-3, 2011/50740–5, and 2013/10498-6), Brazil.

Statement of Interest

Dr Noto has received a scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Dr Gadelha was on the speakers' bureau and/or has acted as a consultant for Janssen-Cilag in the last 12 months and has also received research support from Brazilian government institutions (CNPq). Dr Bressan has received research funding from FAPESP, CNPq, CAPES, Fundação Safra, Fundação ABADS, Janssen, Eli Lilly, Lundbeck, Novartis, and Roche, has served as a speaker for Astra Zeneca, Bristol, Janssen, Lundbeck, and Revista Brasileira de Psiquiatria, and is a shareholder of Radiopharmacus Ltda and Biomolecular Technology Ltd. Dr Maes is supported by a CNPq (Conselho Nacional de Desenvolvimento Cientifico e Technologia) PVE fellowship at the Health Sciences Graduate Program, Londrina State University (UEL). The other authors have no conflicts of interest to disclose.

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