Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Activation of the Immune-Inflammatory Response System and the Compensatory Immune-Regulatory Reflex System in Antipsychotic Naive First Episode Psychosis

Mariane Nunes Noto [a], Michael Maes [b], Sandra Odebrecht Vargas Nunes [c], Vanessa Kiyomi Ota [d], Ana C. Rossaneis [e], Waldiceu A. Verri Jr [e], Quirino Cordeiro [f], Sintia Iole Belangero [d], Ary Gadelha [a,g], Rodrigo Affonseca Bressan [b,*], Cristiano Noto [a,g,*]

[a] GAPi (Early Psychosis Group), Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil

[b] Department of Psychiatry, Chulalongkorn University, Bangkok, Thailand

[c] Health Sciences Graduate Program, Health Sciences Center, State University of Londrina (UEL), Londrina, Brazil

[d] Genetics Division, Department of Morphology and Genetics, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil

[e] Department of Pathology, Biological Sciences Center, State University of Londrina (UEL), Londrina, Brazil

[f] Department of Psychiatry, Faculdade de Ciências Médica da Santa Casa de São Paulo (FCMSCSP), São Paulo, Brazil

[g] Schizophrenia Program (PROESQ), Department of Psychiatry, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil

Corresponding author

Dr. C. Noto

© 2018 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Rua Pedro de Toledo, 669, 3º andar

CEP 04039-032, São Paulo/SP, Brasil

Phone: +55 11 5576-4845

E-mail: csnoto@gmail.com

* These authors share senior authorship

Running Title: Immune-inflammatory biomarkers in FEP

Number of words in abstract: 300

Number of words in article body: 5109

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

ABSTRACT

Background: First episode psychosis (FEP), schizophrenia and affective disorders are accompanied by activation of the immune inflammatory response system (IRS). The compensatory immune-regulatory reflex system (CIRS) is a regulatory immune response that is induced by the IRS but exerts negative feedback through, for example, increased levels of anti-inflammatory cytokines such as IL-4, IL-13 and IL-10. Different phenotypes of schizophrenia may exhibit distinct IRS and CIRS immune profiles.

Aims: This study aims to examine the IRS and CIRS components, including macrophagic M1, T-helper (Th)-1, Th-2, Th-17 and T-regulatory (Treg) phenotypes, in antipsychotic-naïve FEP patients before and after risperidone treatment.

Methods: We included 31 antipsychotic-naïve FEP patients who had measurements of IRS and CIRS biomarkers before and after treatment with risperidone for 10 weeks, and 22 healthy controls.

Results: Antipsychotic-naive FEP patients showed interrelated increments in M1, Th-1, Th-2, Th-17 and Treg phenotypes and a relatively greater IRS response (especially granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6 and IL-12) as compared with the CIRS response (IL-4, IL-13, IL-5 and IL-10). Inflammatory markers, especially IL-6 and IL-8, were significantly correlated with negative, psychotic, affective and excitation symptom dimensions. Treatment with risperidone significantly suppressed the IRS and CIRS. Baseline levels of CIRS biomarkers, especially higher soluble tumor necrosis factor receptor-1 and IL-10 predicted clinical improvement during treatment.

Discussion: Our findings indicate that FEP is characterized by robust IRS (M1 + Th-1 + Th-17) and CIRS responses, suggesting that monocytes, macrophages, Th-1, Th-2,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Th-17 and Treg cells are activated. The findings indicate that a) FEP patients are prone to the detrimental effects of M1, Th-1, Th-17 and Th-2 cells, which may contribute to long-lasting abnormalities in brain circuitry; and b) in FEP, the CIRS may contribute to recovery from the acute phase of illness. Enhancing the CIRS is a new drug target to treat FEP.

Keywords: schizophrenia; first episode psychosis; antipsychotic; immune; inflammation; cytokines

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

INTRODUCTION

There is robust evidence that severe mental disorders, including schizophrenia and bipolar disorder (BD), are characterized by an activation of the immune-inflammatory response system (IRS) [1-5]. The IRS may induce alterations in different components of the compensatory immune-regulatory reflex system (CIRS), including increased production of negative immune-regulatory or anti-inflammatory cytokines including IL-4, IL-10 and transforming growth factor(TGF)-ˆ, which in turn may downregulate the primary IRS response [6,7].

Meta-analyses demonstrated that a pro-inflammatory syndrome is present in schizophrenia and BD [1,4,5]. While some cytokines such as IL-1 β, IL-6, and transforming growth factor (TGF)- β may be altered during acute exacerbations (state markers), other cytokines (or receptors) including IL-12, IFN- γ, TNF- α, and sIL-2R may be considered to be trait markers of schizophrenia [1]. Alterations in cytokine profiles may be present since the early stages of psychosis, even before antipsychotic treatment. Recent studies showed significant elevations in pro-inflammatory cytokine (and receptor) levels (IL-1 β, sIL-2r, IL-6, and TNF- α) in antipsychotic naive first-episode psychosis (FEP) patients [8,9]. Affective disorders are accompanied by M1 macrophagic activation; increased production of tumor necrosis factor alpha (TNF)- α, interleukin (IL)-1 β, IL-6 and soluble IL-6 receptor (sIL-6R); and T helper (Th)-1 and Th-17 responses with increased levels of IL-2 and interferon gamma (IFN)- γ and IL-17, respectively [6,5].

Macrophages, monocytes and T cells exist in functionally distinct phenotypes including M1 macrophages (producing IL-1, IL-6 and TNF- α), and Th1 (associated with increased IL-2, IL-12, IFN- γ and sIL-2R levels), Th2 (producing IL-4, IL-5 and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

IL-13) and Th-17 (producing IL-17), cells and T regulatory (Treg) cells (producing TGF- β - and IL-10) [10]. M1, Th-1 and Th-17 are generally pro-inflammatory, while Th2 and Treg have regulatory functions [6].

Antipsychotic drugs have immunoregulatory effects as indicated by significant alterations in cytokine levels during antipsychotic treatment [11-15]. Nevertheless, inconsistent results on cytokine alterations after antipsychotic treatment are found among studies. A recent meta-analysis on cytokine levels after antipsychotic treatment in antipsychotic-naïve FEP subjects found that IL-6 and IL-2, and possibly IL-1 β, could be considered state markers, which decreased during antipsychotic treatment, whilst TNF- α, IL-17, and IFN- γ might be considered trait markers [16]. A previous study, performed by our group, showed a decrease in IL-4, IL-6, IL-10 and TNF- α levels during risperidone treatment, suggesting immunoregulatory effects of this drug, characterized by suppressant effects on macrophagic M1, Th-2 and Treg functions [14,15].

The aim of this study was to delineate M1, Th-1, Th-2, Th-17 and Treg phenotypes and the IRS / CIRS ratio in antipsychotic-naïve FEP patients both before and during treatment with risperidone and to examine whether the activated CIRS functions may predict a better clinical outcome. Secondary outcomes of the study were to compare differences in those biomarker profiles between schizophrenia and BD, and associations with symptom severity and treatment response to risperidone in FEP patients.

METHODS

This study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Research Ethics Committee of UNIFESP (Sao

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Paulo, Brazil), and all participants provided written informed consent prior the enrollment (CEP nº 0729/15).

Participants

We recruited antipsychotic-naïve FEP patients (n=31) admitted to a psychiatric emergency unit in São Paulo, Brazil. For the purpose of the study, FEP was defined as 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 the diagnoses of schizophrenia (n=16) or bipolar disorder (n=15) according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), using the Structured Clinical Interview of the DSM-IV (SCID-I). We included individuals between 16 and 40 years old who had never used any antipsychotic drugs before admission. Exclusion psychiatric criteria were psychotic episodes due to a general medical condition, substance-induced psychotic disorder and intellectual disability. 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 and immunological disorders. 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 titration was performed according to clinical judgment. Other medications were used in the period between both assessments, according to symptomatology.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

The comparison group consisted of antipsychotic-naïve, healthy volunteers (n=22), assessed for personal and familiar history of psychiatric disorders, according to the criteria of the SCID-I. Only individuals without current or lifetime history of psychiatric disorders and absence of a major mental disorder in first-degree relatives were included. The general exclusion criteria were the same of the individuals with FEP.

The relative severity of illness dimensions was assessed using the Positive and Negative Syndrome Scale (PANSS) [17]. Response to treatment was defined as a reduction of more than 50% of baseline PANSS total score [18]. 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 [18].

Methods

Blood samples (10 mL) were withdrawn from all patients at admission, before the first dose of risperidone and after 10 weeks of treatment and from all healthy controls. Blood was immediately centrifuged at 1,300 g for 10 min and the serum was stored at −80◦C until thawed for assays of cytokines. All samples were thawed the same day and assays were carried out in one and the same run using the same batch of reagents. The concentrations of IL-1β: interleukin 1 beta; IL-10: interleukin 10; IL-13: interleukin 13; IL-6: interleukin 6; IL-7: interleukin 7; IL-15: interleukin 15; IL-5: interleukin 5; IL-12: interleukin 12; IL-1ra: interleukin 1 receptor antagonist; IL-2: interleukin 2; IL-17: interleukin 17; sIL-2R: interleukin 2 receptor; IL-4: interleukin 4; IL-8: interleukin 8; GM-CFS: granulocyte-macrophage colony-stimulating factor; IFN-γ: interferon gamma; TNF- α : tumor necrosis factor alpha; Soluble TNF-receptor 1 (sTNF-R1), Soluble TNF- receptor 2 (sTNF-R2), in the serum of patients

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

were determined by Luminex MAGPIX® system assay. Immediately prior to it, the samples were clarified by an additional centrifugation for 10 min at 12,000 g. The assays were performed according to instructions of the manufacturer. Briefly, samples were diluted in assay diluent, and the reconstituted lyophilized standard of analyte was diluted for the standard curve in a 96-well plate. The diluted capture bead solution was added and followed by a wash. The incubation buffer and standard were added, and the plate was covered and incubated for 2 hours at room temperature on an orbital plate shaker. After a new wash, the diluted biotinylated detector antibody was added, and the plate was covered and incubated for one more hour followed by another wash. The streptavidin-RPE was added and incubated for 30 minutes, then the plate was washed and read on the MAGPIX instrument. The results are expressed as picograms (pg) of biomarker per ml of serum.

Statistical analyses

Analysis of variance (ANOVA) and analysis of contingency tables (X^2 test) were used to examine differences in socio-demographic and clinical data between the study samples. Multivariate general linear model (GLM) analyses were used to examine the effects of explanatory variables (diagnostic groups and socio-demographic data) on cytokines levels while adjusting for age and sex. When the multivariate analysis yielded significant effects, we used tests for between-subject effects or univariate GLM analyses to assess effects of the significant explanatory variables (diagnosis) on cytokines. Consequently, estimated marginal mean values (SE) were computed and protected posthoc analyses were used to assess the differences between controls and FEP. Results of multiple comparisons were p-corrected for false discovery rate (FDR) according to Benjamini and Hochberg (1995)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

[19]. Effects of treatments were assessed using Linear Mixed Model (LMM) analysis, repeated measurements, to examine the effects of treatment, while adjusting for age and sex. Relationships between variables were assessed by means of Pearson's product moment and Spearman rank-order correlation correlations. Multiple regression analysis was used to examine the effects of cytokines on severity of illness. The PANSS total scores, positive, negative and general subscales were used in the analyses. Moreover, three specific subscales were computed reflecting psychotic symptoms (psychosis), excitement-hostility, and anxiety-depression (mood). These subscales were validated by their latent constructs displaying adequate composite reliability, Cronbach's alpha, rho_A and R^2 values obtained in this study sample and other larger study groups (data not shown). Thus, the sum of P1 (delusions), P2 (conceptual disorganization), P3 (hallucinations), P6 (persecution) and G9 (unusual thought content) were used as an index of psychosis. The sum of P4 (excitement), P7 (hostility), G8 (uncooperativeness) and G14 (poor impulse control) reflected excitement-hostility. The sum of G2 (anxiety), G4 (tension). G3 (guiltfeeling), G6 (depression) and G7 (motor retardation) were used as a mood construct. Cytokine data were ln-transformed and, consequently, z scores (a distribution with mean=0 and SD=1) were computed. The intergroup mean z score (SE) values of all cytokines and composite scores are displayed in Figures. Statistical analyses levels were performed using IBM SPSS, Windows version 24. Statistical significance was set at alpha ≤ 0.05 (two tailed).

Based on the known cytokine profiles of M1, Th-1, Th-2, Th-17 and Treg and CIRS cytokines we have computed z unit weighted composite scores which represent different immune profiles [6]:

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

1) The macrophagic M1 profile was computed as z value of interleukin (IL)-6 (zIL-6) + zTNF- α + zGM-CSF (granulocyte and macrophage colony -stimulating factor);

2) M1 + Th-17 profile was computed as M1profile+ zIL-17;

3) Th1 profile was computed as zIFN-$\smile$ + zlL-12 + zIL-2 + zsIL-2R);

4) All.IRS (all cytokines of the IRS) was computed as a z composite score of all cytokines belonging to M1, Th1 and Th17 (all except sIL-2R)

5) Th2 + Treg profile was computed as zIL-4 + zIL-5 + zIL13 + zIL-10;

6) CIRSr (sum of receptor levels belonging to the CIRS) was computed as zsIL-2R + zsIL-1RA (sIL-1 receptor antagonist) + zsTNF-R1 (sTNF-receptor 1) + zsTNF-R2

7) All.CIRS (all cytokines and receptors belonging to the CIRS): computed as sum of Th2 + Treg + CIRSr profiles

8) IRS/CIRS ratio was computed as z (All IRS cytokines) – z (all CIRS cytokines and CIRSr).

Firstly, we performed a multivariate GLM analysis with M1 + Th17; Th1; All IRS; Th2 + Treg; CIRSr; All CIRS; and IRS/CIRS ratio as dependent variables and diagnosis as explanatory variable. When significant (after p-correction for FDR), we examined the effects of diagnosis on the cytokine levels belonging to a specific profile (e.g. the M1+Th17 cytokine profile in this GLM analysis was represented by GM-CSF, IL-6, TNF- α and IL-17 levels) and the results were again interpreted after p-correction for FDR. It is also important to note that some variables, e.g. sIL-2R, which in itself an index of T cell activation (Th-1) displays CIRS activities [6].

RESULTS

Socio-demographic data

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Table 1 shows the socio-demographic data of patients and controls in the study. There were no significant differences in age, sex, BMI, self-declared ethnicity, marital status and living arrangement between the two study groups.

Table 1 - Socio-demographic and clinical data in healthy controls (HC) and patients with first episode psychosis (FEP)

Variable	HC	FEP	X^2/F/□	df	p
Age (years)	25.0 (6.7)	25.8 (6.4)	0.21	1/51	0.652
Sex (Male %)	59.1	61.3	0.031	1	0.872
BMI (kg/m^2)	23.9 (3.6)	23.4 (4.7)	0.10	1/32	0.759
Ethnicity (Caucasian %)	81.2	55.6	2.92	1	0.087
Marital Status (single %)	87.5	78.6	0.11	-	0.689
Living arrangement (with parents %)	62.5	60.7	0.03	-	0.997

The intercorrelation matrices among the composite scores showed that in controls and antipsychotic naive FEP patients, there were significant correlations between M1+Th17 and Th2+Treg (r=0.755, p<0.001, n=53) and All.CIRS (r=0.566, p<0.001, n=53) and between Th1 and T2+Treg (r=0.642, p<0.001, n=53) and All.CIRS (r=0.746, p<0.001, n=53) (Table2). All.IRS was significantly associated with All.CIRS (r=0.639, p<0.001, n=53) (Table2). In risperidone-treated patients there were significant correlations between M1+Th17 and Th2+Treg (r=0.636, p<0.001, n=30) and All.CIRS (r=0.471, p=0.009, n=30) and between Th1 and All_CIRS (r=0.624, p<0.001, n=30), but not Th2+Treg (r=0.117, p=0.537, n=30) (Table 4). After treatment there was only a weak correlations (which was no longer significant after p-correction) between All.IRS and All.CIRS (r=0.367, p=0.046, n=30).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Effects of diagnosis on composite scores

Table 2. Results of multivariate GLM analyses with different types of cytokine profiles as dependent variables and diagnosis as primary explanatory variable

Test	Dependent variables	Explanatory variables	F/X2	df	p	p corrections FDR
≠1 Multivariate	7 IRS/ CIRS Variables	Diagnosis	2.43	12/142	0.008	
		Sex	2.08	6/70	0.067	
		Age	0.41	6/70	0.870	
Between Subject Effects	M1 + Th17	Diagnosis	8.93	2/75	<0.001	0.0021
	Th1	Diagnosis	6.02	2/75	0.004	0.0056
	All IRS	Diagnosis	8.99	2/75	<0.001	0.0021
	Th2 + Treg	Diagnosis	10.96	2/75	<0.001	0.0021
	CIRSr	Diagnosis	0.46	2/75	0.632	0.632
	All CIRS	Diagnosis	5.42	2/75	0.006	0.007
	IRS/CIRS	Diagnosis	6.82	2/75	0.002	0.0035
≠2 Multivariate	M1+Th17 Cytokines	Diagnosis	5.24	8/142	<0.001	
Between Subject Analyses	GM-CSF	Diagnosis	8.52	2/75	<0.001	0.0018
	IL-6	Diagnosis	15.00	2/75	<0.001	0.0018
	TNF-α	Diagnosis	3.82	2/75	0.026	0.026
	IL-17	Diagnosis	4.71	2/75	0.012	0.016
≠3 Multivariate	Th1 Cytokines	Diagnosis	2.49	8/144	0.015	
Between Subject Effects	IFN-γ	Diagnosis	8.07	2/75	0.001	0.002
	IL-12	Diagnosis	10.00	2/75	<0.001	0.0025
	IL-2	Diagnosis	0.55	2/75	0.578	0.578
	$_{S}$IL-2R	Diagnosis	1.31	2/75	0.275	0.275
≠4 Multivariate	CIRS	Diagnosis	4.79	14/138	<0.001	
Between Subject Effects	IL-4	Diagnosis	5.15	2/75	0.008	0.011
	IL-5	Diagnosis	9.78	2/75	<0.001	0.002
	IL-13	Diagnosis	10.49	2/75	<0.001	0.002
	IL-10	Diagnosis	8.83	2/75	<0.001	0.002
	$_{S}$IL- IRA	Diagnosis	12.34	2/75	<0.001	0.002
	$_{S}$TNF-RI	Diagnosis	1.85	2/75	0.165	0.193
	$_{S}$TNF-RII	Diagnosis	0.81	2/75	0.449	0.449
≠5 Multivariate	3 Other Cytokines	Diagnosis	3.24	6/146	0.005	
Between Subject Effects	IL-7	Diagnosis	0.91	2/75	0.408	0.408
	IL-15	Diagnosis	5.06	2/75	0.009	0.014
	IL-8	Diagnosis	5.30	2/75	0.007	0.014

* Diagnosis: 3 groups, namely healthy controls and patients with first episode psychosis before and after treatment

All multivariate analyses are adjusted for age and sex

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

MI: MI macrophagic profile cytokines computed as z value of Interleukin (IL)-6 (zIL-6) + zTNFα (Tumor Necrosis Factor)-α + zGM-CSF (granulocyte and macrophage colony - stimulating factor)
M1 + Thelper (Th) 17 profile: computed as M1profile+ zIL-17
Th1 profile: computed as zIFN (interferon-ـ) + zIL-12 + zIL-2 +z soluble IL-2R (zsIL-2 receptor)
Th2 + T reg (T regulatory) profile: computed as zIL-4 + zIL-5 + zIL13 + zIL-10
CIRSr (CIRS receptors functioning as compensatory immune regulatory reflex system) computed as zsIL-2R + zsIL-1RA (sIL-1 receptor antagonist) + zsTNF-RI (sTNF-receptor I) + zsTNF-RII
All IRS (all cytokines of the immune-inflammatory response system: computed as a z composite score of all cytokines belonging to M1, Th1, Th17 and IL-8 (all except sIL-2R)
All CIRS (all cytokine/receptors of the CIRS): computed as sum of Th2 + Treg + CIRSr profiles
IRS/CIRS: computed as z(All IRS) – z (All CIRS)

Table 2 shows the results of multivariate GLM analyses with the 7 composite scores as dependent variables and diagnosis (controls, antipsychotic naive patients and risperidone-treated patients), age and sex as explanatory variables. We found significant effects of FEP versus the control group (but not age and sex) on the composite scores. Tests for between-subject effects showed that (after *p*-correction) there were significant effects of diagnosis on M1+Th17, Th1, All.IRS, Th2+Treg, All.CIRS and IRS/CIRS, but not CIRSr. Figure 1 shows the z transformed values of the different composite scores in the three conditions. Consequently, we have performed pairwise *post-hoc* analyses comparing controls with antipsychotic naive FEP patients and controls with post-treatment patients. We found that (see also Figure 1) antipsychotic naive FEP patients have significantly higher M1+Th1 (p=004), Th1 (p=0.017), all.IRS (*p*=0.008), Th2+Treg (*p*=0.001), and IRS/CIRS (*p*=0.030), but not CIRSr (*p*=0.880) as compared with controls. The post-treatment M1+Th1 (*p*=0.461), Th1 (*p*=0.520), all.IRS (*p*=0.258), Th2+Treg (*p*=0.430), CIRSr (*p*=0.490) and IRS/CIRS (*p*=0.265) did not differ from control values. Entering BMI in multivariate regression #1 (Table 2) shows that BMI has no significant effect on the composite scores (F=0.87.41, df=6/40, *p*=0.523). There were also no correlations between BMI and any of the separate composite scores (even without *p*-correction). In addition,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

there were no significant effects of ethnicity (F=1.61, df=6/60, *p*=0.161) and smoking (F=1.56, df=6/32, *p*=0.191) on the composite scores.

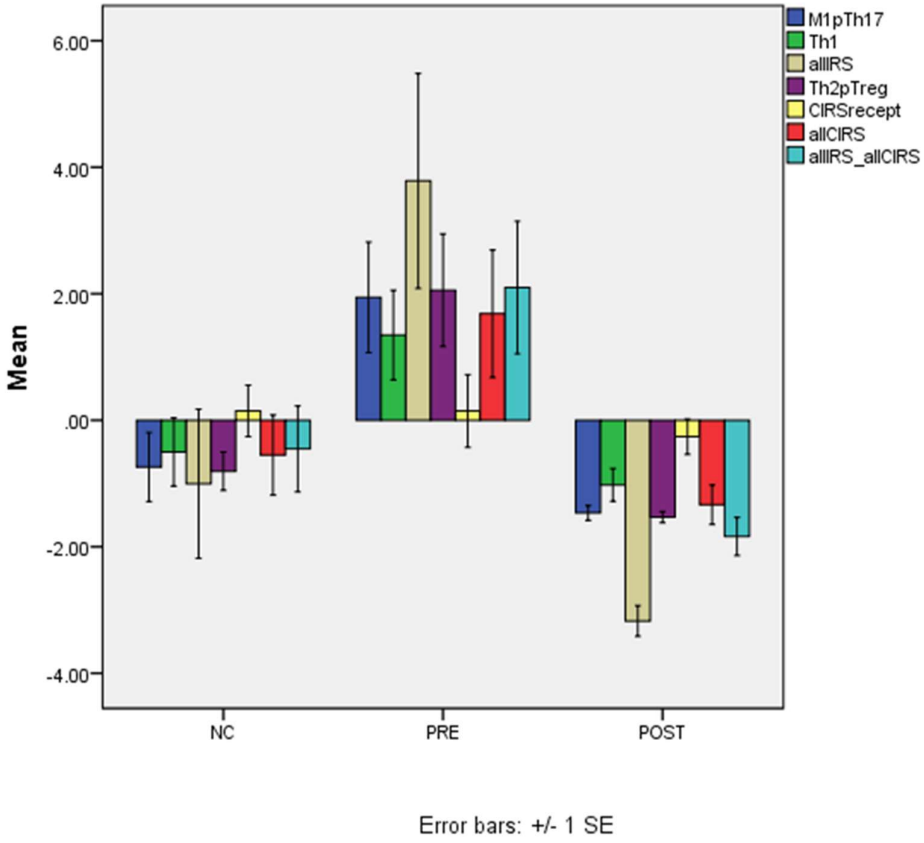

Figure 1 shows the z transformed values of different composite scores in normal controls (NC) and first episode psychosis patients before (PRE) and after (POST) treatment with risperidone. M1pTh17: sum of z scores of cytokines belonging to M1 macrophagic and T helper (Th)-7 phenotypes; Th1: sum of Th-1 phenotype cytokines; allIRS: sum of all cytokines belonging to the immune-inflammatory responses system; Th2pTreg: sum of z scores of cytokines belonging to Th-2 and T regulatory phenotypes; CIRSrecept: sum of receptor levels belonging to the compensatory immune-regulatory reflex system (CIRS); allCIRS: sum of all cytokines and receptors belonging to the CIRS; allIRS_allCIRS: ratio of IRS / CIRS; all data are shown as z scores.

Effects of diagnosis on cytokine and cytokine receptor levels

Consequently, we have examined the different cytokine levels in multivariate GLM analyses. In Multivariate GLM analysis #2 we examined M1+Th17 cytokines (namely GM-CSF, IL-6, TNF- α and IL-17) and detected that diagnosis had a

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

significant effect on these four cytokines. Figure 2 shows the z transformed values of the M1+Th17 cytokines, while Table 3 shows the model-generated estimated marginal means in the three study groups. *Post-hoc* analyses showed higher IL-6 and GM-CSF in antipsychotic naive FEP patients than in controls. There were no significant differences between the M1 and Th17 cytokines among post-treatment FEP patients and controls.

Table 3. Model-generated estimated marginal means of the cytokine (-receptor) levels in healthy controls (HC) and patients with first episode psychosis before (pre) and after (post) treatment with risperidone

Cytokine	Type	IRS/CIRS	HC	Pre	Post
IL-6	M1	IRS	1.47(0.34)[b]	3.08 (0.29)[a]	1.46 (0.31) +
TNF-α	M1	IRS	3.80 (0.26)	4.12 (0.22)	0.37 (0.23) +
IL-17	Th-17	IRS	10.82 (1.09)	12.33 (0.92)	9.29 (0.97) +
GM-CSF	M1	IRS	7.11 (0.53)[b]	9.11 (0.45)[a]	6.99 (0.47) +
IFN-γ	Th-1	IRS	4.97 (0.53)[b]	7.03(0.45)[a]	4.93 (0.471) +
IL-12	Th-1	IRS	11.06 (4.59)[b]	28.74 (3.90)[a]	10.98 (4.09) +
IL-2	Th-1	IRS	2.43 (0.71)	2.34 (0.61)	1.59 (0.64) -
sIL-2R	Th-1	CIRSr	132.2 (32.6)	123.1 (27.6)	81.2 (29.0) +
IL-4	Th-2	CIRS	13.36 (2.64)	13.75 (2.24)	8.18 (2.35) +
IL-5	Th-2	CIRS	2.60 (0.88)[b]	6.20 (0.74)[a]	2.67 (0.78) +
IL-13	Th-2	CIRS	5.48 (0.43)[b]	7.37 (0.37)[a]	5.51 (0.39) +
IL-10	Treg	CIRS	6.44 (0.27)[b]	7.20 (0.23)[a]	6.02 (0.24) +
sIL-1RA	M1	CIRSr	174.96(35.70)[c]	263.53(30.30)	119.02 (31.79)[a] -
sTNF-RI	M1/Th-1	CIRS	701.2 (73.6)	735.15 (62.4)	875.36 (65.5)-
sTNF-RII	M1/Th-1	CIRS	3064 (261)	2883 (221)	3290 (232) -
IL-7	-	-	9.53 (0.86)	9.05 (0.73)	8.47 (0.76)
IL-15	-	-	2.60 (0.88)[c]	6.20 (0.74)	0.26 (0.78)[a]
IL-8	-	-	7.60 (1.62)	9.78 (1.38)	5.27 (1.45)

M1: macrophage M1 profile

Th-1: T helper-1 profile

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Th-2: T helper- 2 profile

Treg: T regulatory profile

CIRS: Compensatory immune regulatory reflex system

IRS: Immune inflammatory response system

For other abbreviations: see legend to table 2

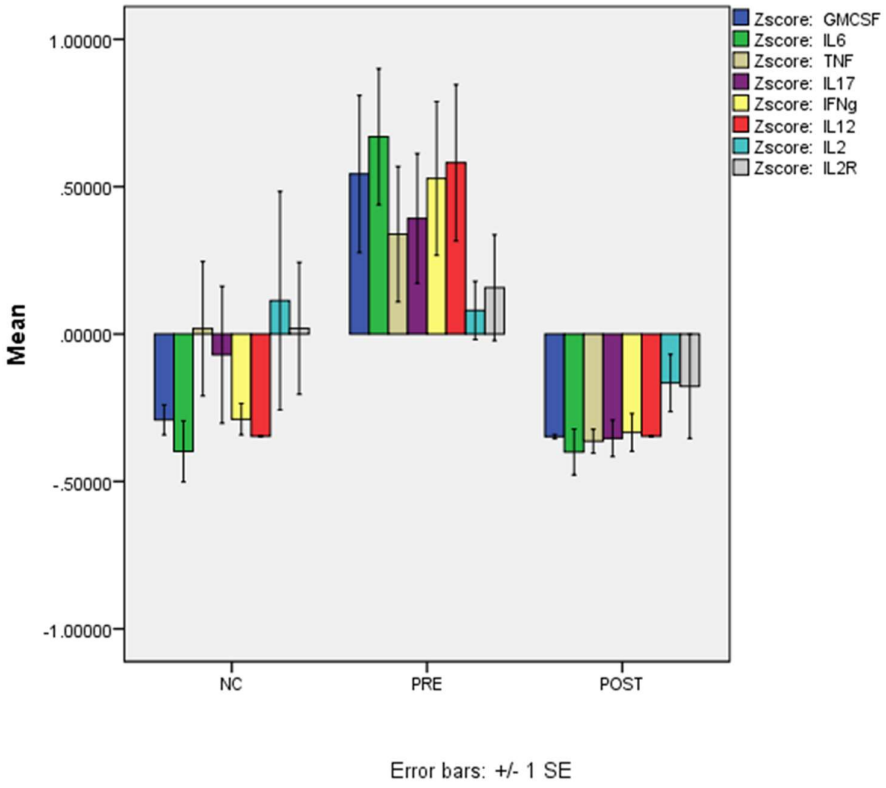

Figure 2 shows the z transformed values of macrophagic M1 and T helper (Th17) cytokine levels assayed in normal controls (NC) and first episode psychosis patients before (PRE) and after (POST) treatment with risperidone, including granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL6), tumor necrosis factor-α (TNF), interferon-γ (IFNg) and the soluble IL-2 receptor (IL2R).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Multivariate analysis #3 examined Th1 cytokines and receptors (namely IFN γ, IL-12, IL-2 and sIL-2R) and showed that diagnosis had a significant effect on these cytokines. Figure 2 shows the z transformed values of these four cytokines, while Table 3 shows the model-generated estimated marginal means. *Post-hoc* analyses showed significantly higher IFN-γ and IL-12 values in antipsychotic naive FEP patients than in controls, whilst there were no significant differences in Th1 cytokines between the post-treatment FEP patients and controls.

Multivariate analysis #4 examined CIRS cytokines and receptors (namely IL-4, IL-5, IL-13, IL-10, sIL-1RA, sTNF-R1 and sTNF-R2). The multivariate analysis showed that there was a significant effect of diagnosis. Figure 3 shows the z transformed values and Table 3 shows the model-generated estimated marginal means of the CIRS cytokines/receptor levels. IL-5, IL-13 and IL-10 were significantly higher in antipsychotic naive FEP patients. Post-treatment sIL-1Ra values were significantly lower than in the control condition.

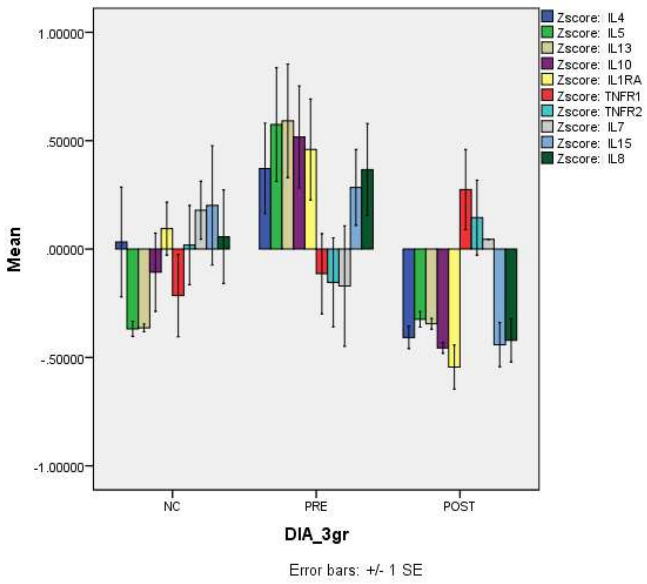

Figure 3 shows the z transformed values of cytokines and receptors belonging to the compensatory immune-regulatory reflex system (CIRS) in normal controls (NC) and first episode psychosis patients before (PRE) and after (POST) treatment

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

with risperidone, including interleukin (IL)-4, soluble IL-1 receptor antagonist (IL-1RA), soluble tumor necrosis factor receptor-1 and -2 (TNFR1 and TNFR2). This figure also shows the z values of IL-7, IL-15 and IL-8.

Finally, we have examined the effects of diagnosis on the three cytokines not classified in a specific profile, namely IL-7, IL-15 and IL-8. Figure 3 (unadjusted means values) and Table 3 (adjusted mean values) show that there were no significant differences between antipsychotic naive FEP patients and controls in these three cytokines, whilst post-treatment IL-15 values were significantly lower than control values.

Effects of risperidone on the composite scores and rating scales

We used LMM analysis to examine the effects of risperidone on the composite scores, using the latter as dependent variables and treatment, age and sex as explanatory variables. Figure 1 shows the pre- and post-treatment values in the FEP patients. We found that treatment with risperidone significantly decreased (after *p*-correction was made) All.IRS, All.CIRS, IRS/CIRS, M1, Th1 and Th2 values. Nevertheless, there were no significant effects of risperidone in CIRSr levels.

Using LMM we also examined the effects of treatment on rating scale scores after adjusting for age and sex. Figure 4 shows the differences in z-transformed values of the rating scale scores between the pre- and post-treatment condition. We found that (see Table 4) risperidone significantly decreased PANSS total, PANSS negative, PANNS positive and PANSS general scale scores. There were also significant effects of risperidone on the excitement, psychosis and mood scores.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

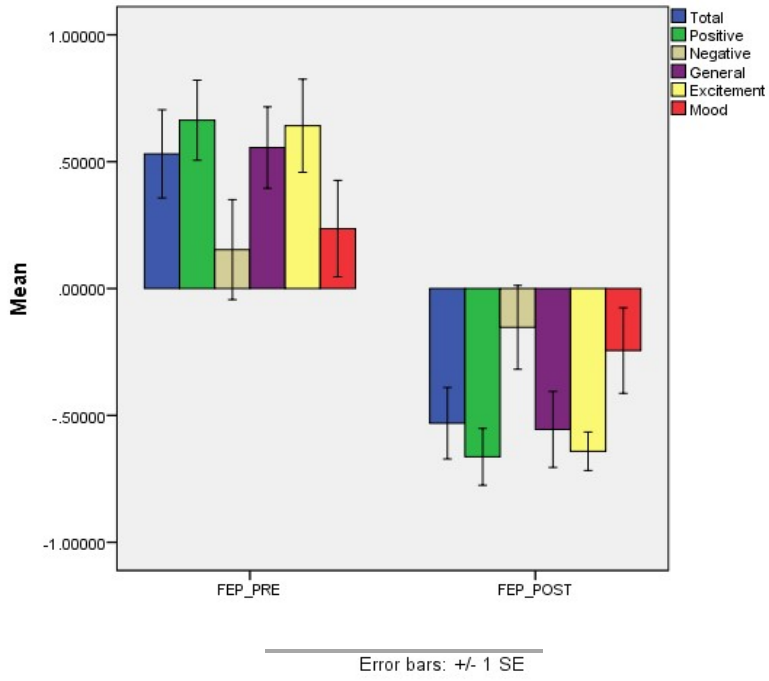

Figure 4 shows the differences in z-transformed values of the Positive and Negative Syndrome Scale (PANSS) rating scale scores in first episode psychotic (FEP) patients before (FEP_PRE) and after (FEP_POST) treatment with risperidone. Shown are effects on the z-transformed scores of the total PANSS score, positive, negative and general subscales as well as excitement-hostility and affective (mood) subscales.

Table 4. Effects of treatment with risperidone on the immune-inflammatory response system (IRS) and the compensatory immune-regulatory reflex system (CIRS) and symptom profiles

Variables	Pre-treatment	Post-treatment	Wald	df	p	FDR
All IRS/ All CIRS	+2.04 (1.01)	-1.86 (0.36)	13.18	1	<0.001	0.0013
All IRS (z scores)	+3.80 (1.69)	-3.16 (0.29)	17.00	1	<0.001	0.0013
All CIRS (z scores)	+1.76 (0.99)	-1.29 (0.38)	9.45	1	0.002	0.0023
CIRSr (z scores)	+0.24 (0.55)	-0.21 (0.33)	0.61	1	0.434	0.434
M1 (z scores)	+1.74 (0.67)	-1.22 (0.14)	17.36	1	<0.001	0.0023
Th-1 (z scores)	+1.76 (0.70)	-1.05 (0.30)	13.96	1	<0.001	0.0023
Th-2 (z scores)	+1.71 (0.73)	-1.22 (0.17)	13.01	1	<0.001	0.0023
PANSS total	94.42 (5.09)	60.77 (4.26)	28.27	1	<0.001	0.0013
Negative	20.59 (1.71)	17.45 (1.32)	5.01	1	0.025	0.025

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Positive	26.24 (1.46)	12.93 (1.05)	40.50	1	<0.001	0.0013
General	46.41 (2.73)	30.06 (1.98)	30.80	1	<0.001	0.0013
Excitement hostility	12.47 (0.94)	5.67 (0.38)	49.03	1	<0.001	0.0013
Psychosis	19.72 (1.09)	10.29 (0.94)	50.75	1	<0.001	0.0013
Mood	12.34 (1.03)	9.85 (0.79)	5.08	1	0.024	0.025

For exaplanation of the cytokine profiles: see legends to Table 2

Associations between cytokine levels and rating scale scores

Using regression analyses, we have analysed the associations between cytokines (and age and sex) and rating scale scores or Δ differences (pre - post-treatment) in the rating scale scores. Inspection of the correlation matrices showed that IL-6, IL-8, IL-10 and CIRSr including sTNF-R1 showed consistent associations with the rating scale scores. Therefore, we have entered these 5 cytokine values in multiple regression analyses with the rating scales and the Δ differences (pre - post-treatment values) as dependent variables. Table 5 shows the results of these regression analyses. We found that 29.4% of the variance in the PANSS total score was explained by IL-6 (positively) and age. 35.6% of the variance in PANSS positive was explained by IL-6, IL-8 and age. 25.0% of the variance in PANSS negative score was associated with IL-6 and male sex, while IL-6 alone explained 16.1% of the variance in PANSS general score. Excitement-hostility was significantly predicted by IL-6, IL-8 (both positively) and age (negatively). Psychosis scores were predicted by IL-6 explaining 18% of the variance, whilst IL-8 levels were associated with mood scores. IL-6 was also significantly associated with baseline PANSS total ($F=4.84$, $df=1/27$, $p=0.037$, $R^2=15.2\%$), PANSS negative ($F=8.36$, $df=1/28$, $p=0.007$, $R^2=23.0\%$) and excitement-hostility ($F=7.93$, $df=1/28$, $p=0.009$, $R^2=22.1\%$) scores, whilst IL-8 was associated with basal PANSS positive scores ($F=6.20$, $df=1/28$, $p=0.019$, $R^2=18.1\%$). The Δ values on the other hand were significantly associated with CIRS variables.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Thus, a large part of the variances in Δ PANSStotal (27.5%), Δ PANSS general (26.3%) and Δ PANSS mood (16.7%) were explained by basal CIRSr values. Basal IL-10 levels were positively associated with Δ PANSS negative (explaining 14.8% of the variance), while basal sTNF-R1 levels were associated with Δ PANSS psychosis (37.5% of the variance). IL-10 and sTNF-R1 levels together explained 36.8% of the variance in Δ PANSS positive scores and 33.5% in the Δ PANSS excitement-hostility scores.

Table 5. Associations between cytokines (receptor) levels or cytokines profiles and schizophrenia phenomenology

Dependent Variable	Explanatory Variable	t	p	F	df	p	R2 (%)
PANSS total	IL-6	+4.31	<0.001	11.06	2/53	<0.001	29.4
	Age	-2.10					
PANSS positive	IL-6	+2.86	0.006	9.93	3/54	<0.001	35.6
	IL-8	+2.40	0.020				
	Age	-2.30	0.025				
PANSS negative	IL-6	+3.47	0.001	9.16	2/55	<0.001	25.0
	Male Sex	+2.45	0.018				
PANSS general	IL-6	+3.22	0.002	10.37	1/54	0.002	16.1
Excitement	IL-6	+3.17	0.003	11.65	3/54	<0.001	39.3
	IL-8	+2.63	0.011				
	Age	-2.19	0.033				
Psychosis	IL-6	+3.48	0.001	12.08	1/55	<0.001	18.0
Mood	IL-8	+2.47	0.017	6.08	1/55	0.017	10.0
Δ PANSS Total	CIRSr	+3.14	0.004	9.86	1/26	0.004	27.5
Δ PANSS Negative	IL-10	+2.21	0.036	4.86	1/28	0.036	14.8
Δ PANSS Positive	sTNF-RI	+3.64	0.001	7.58	2/26	0.003	36.8
	IL-10	+2.10	0.046				
Δ PANSS Excitement	sTNF-RI	+3.03	0.005	6.81	2/27	0.004	33.5
	IL-10	+2.57	0.016				

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

Δ PANSS Psychosis	sTNF-RI	+4.02	<0.001	16.19	1/27	<0.001	37.5
Δ PANSS General	CIRSr	+3.05	0.005	9.30	1/26	0.005	26.3
Δ PANSS Mood	CIRSr	+2.33	0.028	5.43	1/27	0.028	16.7

IL-6: interleukin-6
CIRSr: CIRS receptors functioning as compensatory immune regulatory reflex system and computed as zsIL-2R (IL-2 receptor) + zsIL-1RA (sIL-1 receptor antagonist) + zsTNF-RI (sTNF-receptor I) + zsTNF-RII

Differences between schizophrenia and bipolar FEP patients

Table 6 shows the mean values of the rating scale scores in schizophrenia and BD FEP patients, while Figure 5 shows the z-transformed values of the rating scale scores. After FDR p-correction, we found that PANSS total, PANSS negative, PANSS general, and mood were significantly higher in schizophrenia FEP than in BD FEP patients. Figure 6 shows the different composite scores in schizophrenia versus bipolar FEP. This figure shows higher M1+Th17, Th1, All.CIRS, Th2+Treg, CIRS receptors, and All.CIRS scores in FEP patients with schizophrenia versus BD and controls. Pairwise comparisons showed significantly higher (p=0.021 after p-correction) M1+Th17, Th1, All.CIRS, Th2+Treg and All.CIRS in schizophrenia FEP patients than in controls, while there were no significant differences between BD FEP patients and controls and between BD and schizophrenia FEP patients.

Table 6 - Differences between FEP patients classified as having schizophrenia or bipolar disorder (BD)

Variables	Schizophrenia	BD	F	df	p	FDR
PANSS total	107.5 (6.1)	82.0 (6.6)	8.12	1/22	0.009	0.021
Negative	26.4 (2.2)	15.3 (2.4)	11.17	1/22	0.003	0.021
Positive	28.0 (2.2)	26.4 (2.5)	0.22	1/22	0.643	0.651
General	53.2 (3.6)	40.4 (4.0)	5.70	1/22	0.026	0.0416
Excitement	12.9 (1.4)	13.9 (1.5)	0.21	1/22	0.651	0.651
Psychosis	21.7 (1.6)	17.7 (1.7)	2.89	1/22	0.103	0.144
Mood	15.3 (1.4)	9.3 (1.5)	8.75	1/22	0.007	0.021

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

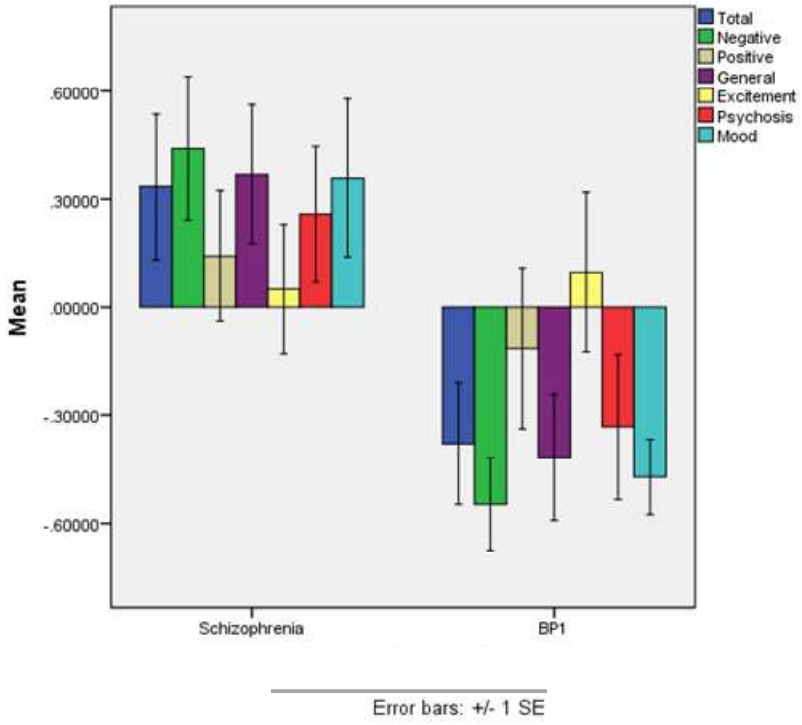

Figure 5 shows the z scores of baseline Positive and Negative Syndrome Scale (PANSS) rating scale scores in first episode psychotic (FEP) patients divided into those who develop schizophrenia versus bipolar disorder type 1 (BP1). Shown are effects on the z-transformed scores of the total PANSS score, positive, negative and general subscales as well as excitement-hostility and affective (mood) subscales.

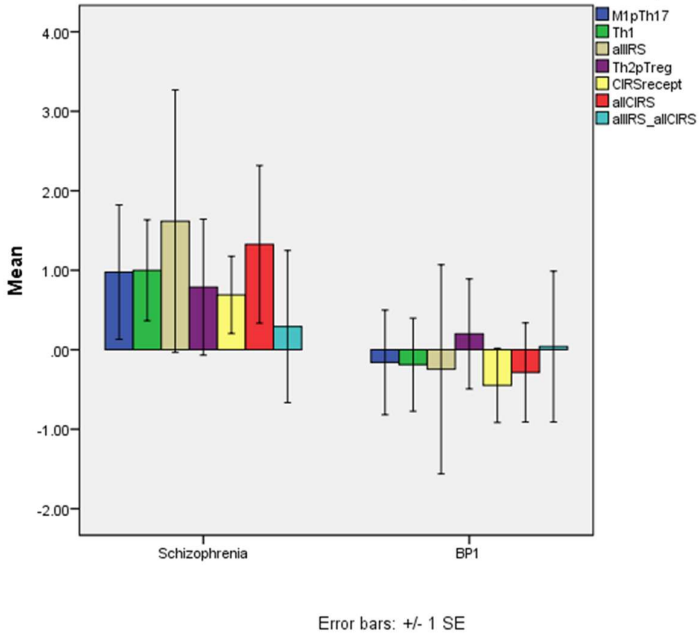

Figure 6 shows the z scores of different composite scores in first episode psychotic patients divided into those who develop schizophrenia versus bipolar disorder type 1 (BP1). See legends to Figure 1 for explanations.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

DISCUSSION

The first major findings of this study are that a) antipsychotic-naïve FEP patients show increased M1, Th-1, Th-2, Th-17 and Treg responses and interrelated elevations in IRS and CIRS responses as compared with normal controls; and b) FEP is characterized by an increased IRS (M1 + Th-1 + Th-17) / CIRS (Th-2 + Treg) response ratio and thus by an immune-inflammatory response. The most significant cytokines separating FEP from controls were GM-CSF, IL-6 and IL-12, representing IRS activation, and IL-4, IL-13, IL-5 and IL-10, representing CIRS activation. Already in the 1990's, it was described that bipolar disorder and schizophrenia are accompanied by an immune-inflammatory response as indicated by increased M1 (IL-6, sIL-1RA), Th-1 (sIL-2R) and Treg (IL-10) cytokines/receptors in schizophrenia and M1 (IL-6) and Th-1 (sIL-2R) in BD, and increased acute phase proteins and complement factors in both disorders [12,20-22]. Our results are also in accordance with previous studies indicating cytokine profiles in FEP patients reflecting activated IRS (IL-2, IL-1 β, IL-6 and TNF- α) and CIRS (IL-4, IL-5, IL-13, IL-10, sIL-1RA, sTNF-R1 and sTNF-R2) [1,8,12,23]. For example, we reported that FEP is accompanied by increased IL-6, TNF- α and IL-10 levels, indicating M1 and Treg activation [9], while another report found increased IL-1 β, IL-6, TNF- α and sIL-2R levels in medication-naïve FEP [8]. Previous reports concluded that patients with psychosis may have an impaired production of Th-1 cytokines and an overactivation of the Th-2 system, leading to a dysfunction in the normal Th1/Th2 balance [24]. Nevertheless, our results using z weighted composite scores computed on a greater number of cytokines belonging to different profiles clearly indicate a more generalized activation of the immune system in FEP.

The cytokine profile observed here may suggest that monocytes, macrophages,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

dendritic cells (DCs), and Th-1 cells might be induced by bacterial or viral infections thereby producing a broad array of cytokines [25,26]. Increases in IL-12, TNF- α, IL-8, IL-4 and IL-10, as detected here in FEP patients, may well indicate the presence of an infection [26]. Moreover, the immune cell profile observed in FEP patients may indicate induction of DCs and macrophages, which subsequently activate Th-1 cells [27]. The lack of an IL-2 response coupled with strongly induced M1 cytokines in FEP may suggest that perhaps a bacterial component is more prominent than a viral component [26]. Nevertheless, other etiological factors cannot be ruled out, including complement activation, although increased complement levels in schizophrenia are probably a consequence of an acute phase response [22].

Moreover, in the present study we found a highly significant correlation between IRS and CIRS activation in FEP individuals. Thus, our findings indicate a strong M1 + Th-1 + Th-17 response coupled with an increased production / secretion of immune mediators that are released during IRS activation but that have negative immune-regulatory or anti-inflammatory effects (CIRS) [6]. We have discussed somewhere else that mood disorders, including bipolar disorder and mania, are accompanied by a primary IRS and a secondary CIRS response, which is induced by the IRS and regulates the IRS [6].

Most important, the findings of the current study indicate that FEP patients are subject to many detrimental effects of M1, Th-1 and Th-17 cytokines. M1 cytokines (including IL-1, IL-6 and TNF- α) and Th-1 cytokines (including IFN- γ) have well established detrimental effects on neuronal functions including neurotransmitter activity, neuroplasticity, apoptosis, neurogenesis and memory [28]. Moreover, in FEP, increased IL-6 may dysregulate *DROSHA* thereby possibly dysregulating cellular functions and the microRNA machinery [29]. Activated Th-1 coupled with M1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

activation may stimulate indoleamine 2,3-dioxygenase (IDO) and kynurenine 3-monooxygenase [30] leading to increased production of excitotoxic or neurotoxic tryptophan catabolites (TRYCATs), including xanthurenic acid, picolinic acid and quinolinic acid and a relative decrease in more protective TRYCATS, such as kynurenic acid [31]. GM-CSF is strongly implicated in activation of "inflammatory, monocyte-derived" DCs and as such it is implicated in inflammatory disease [32]. IL-17 is a pro-inflammatory cytokine that has neurotoxic properties in the brain as observed in patients with multiple sclerosis and stroke [33,34]. IL-8 is another pro-inflammatory cytokine/chemokine that facilitates BBB migration of leukocytes and may sustain brain neuroinflammation resulting in neurotoxic effects, although IL-8 also displays some protective effects [35,36]. All in all, it appears that FEP is characterized by a peripheral IRS, which is possibly induced by infections and which may have profound adverse effects on brain neuronal functioning possibly leading to different symptom dimensions and the deficit phenotype and neurocognitive and memory deficits as well [31].

In our study, FEP is also accompanied by activated CIRS functions (including increased levels of IL-10, IL-4, IL-13 and sIL-1RA), which display beneficial and detrimental effects. For example, IL-10 has negative immune-regulatory effects and may increase the expression of important neuron-associated genes in FEP, including myelin basic protein, *NDEL1* and *DISC1*, thereby exerting neuroprotective effects [29]. sIL-1RA levels may inhibit IL-1 pro-inflammatory signaling [37]. However, in our study the IRS cytokines are significantly more increased as compared with the CIRS cytokines and additionally some of the Th-2 cytokines (which are generally described as anti-inflammatory) may have pro-inflammatory effects. For example, IL-4 may prime macrophages and increase IFN- γ production [38]. Both IL-4 and IL-13

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

may augment the neurotoxic effects of reactive oxygen species [39]. IL-5, another Th-2 cytokine that is induced in FEP patients, is produced by microglia and increases the metabolism, proliferation and activation of microglia cells thereby playing a role in neuroinflammation [40]. Finally, also the different CIRS receptors, including sIL-2R, sTNF-R1, sTNF-R2 and sIL-1RA, which generally have strong immune-regulatory effects [6] are not increased in FEP. Therefore, the lack of a substantial increase in these CIRS receptors coupled with the adverse effects of IL-4, IL-13 and IL-5 may favor a stronger M1, Th1 and Th17 response in FEP. Alternatively, it could be that individuals who have a lowered CIRS responsivity to the primary IRS are at an increased risk to develop FEP following immune activation.

The second major finding of this study is that pro-inflammatory cytokines, such as IL-6 and IL-8, are strongly associated with positive and negative symptoms, excitement, psychosis and mood symptoms. Previously, it was reported that serum IL-4 and IL-10 levels correlate with the negative symptoms in antipsychotic naive individuals with first-episode of psychosis [41]. In schizophrenia patients, it was detected that increased levels of noxious TRYCATs (induced by Th-1 and M1 cytokines) are associated with different symptom dimensions of schizophrenia, including negative symptoms, psychosis, excitation and mood symptoms [31]. Moreover, recently we reported that schizophrenia phenomenology consists of two strongly interrelated symptom dimensions: a) PHEMN dimension with psychotic, hostility, excitation, mannerism and negative symptoms and b) DAPS dimension with depressive, anxiety and physio-somatic symptoms [31]. These data show that the previous two-syndrome framework of “positive versus negative symptoms” [42] is not accurate. All in all, the results of this study show that the two key dimensions of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

schizophrenia phenomenology (PHEMN and DAPS) and key symptoms of mania (excitation) are associated with immune-inflammatory mediators.

Most importantly, we found that changes in the severity of the major symptoms profiles from baseline to some weeks later are strongly associated with the CIRS component, especially sTNF-R1 and IL-10 levels. Phrased differently, the CIRS component predicts a subsequent clinical response whereby lowered levels of these CIRS biomarkers are associated with a worse outcome. These findings support the theory that the CIRS may contribute to spontaneous and treatment-induced recovery from the acute phase of illness in mood disorders [6].

Another finding is that IRS and CIRS scores were higher in patients with a diagnosis of schizophrenia spectrum disorder than in bipolar disorder. Few studies have compared immuno-inflammatory biomarkers between FEP patients who develop bipolar disorder or schizophrenia [43]. Maes *et al*. (1995) compared cytokine levels in euthymic bipolar disorder and schizophrenic patients versus healthy controls and found increased IL-6 in schizophrenia compared to bipolar disorder and healthy controls, whilst in the same study, IL-10 was increased in both schizophrenic and bipolar disorder versus controls. Nevertheless, manic patients showed an immunological profile similar to SCZ patients with IL-6 being more specific for schizophrenia and IL-6 and IL-2R for mania [20]. The findings of the current study may suggest that the combined adverse effects of greater M1, Th-1, Th-17 and Th-2 cytokines leads to schizophrenia rather than bipolar disorder.

A third major finding is that treatment with risperidone significantly suppresses the IRS (M1 and Th-1) and CIRS (Th-2/Treg) leading to an overall suppression of the IRS/CIRS ratio. Previously, it was reported that in FEP patients treatment with risperidone suppressed IL-6, IL-10, TNF- α and IL-4 levels highly

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

significantly. Already in the 1990's it was shown that risperidone may increase plasma sIL-2R levels and plasma CC16, a natural anticytokine [44]. While these studies show that risperidone may have *in vivo* immune-regulatory effects, the effects in animal models are less clear. For example, atypical antipsychotics suppress the stimulated production of pro-inflammatory cytokines and enhance that of IL-10 [45,46]. On the other hand, da Cruz Jung *et al*. (2016) observed that high doses of risperidone may activate an in vitro inflammatory response [47], while Sarvari *et al*. (2014) observed that atypical antipsychotic drugs induce inflammatory gene expression in adipocytes thereby priming them towards an inflammatory state [48]. Importantly, our in vivo findings show that risperidone strongly decreases the generally more protective cytokines, including IL-10, sIL-1RA, IL-13, IL-4 and sIL-1RA and thus that risperidone may be not the best drug to treat FEP. A more adequate treatment should selectively target M1 + Th-1 cytokines and/or the CIRS by increasing the production of its most significant protective molecules, namely sTNF-R1 and IL-10. Treatment with antibodies targeting IL-6, IL-12, IFN- γ and CM-CSF or treatments with IL-10 or TNF-R1 should be trialed to treat FEP and thus prevent conversion to schizophrenia or BD. Future research should delineate the causative factors underpinning the IRS response in FEP with a focus on the possible role of bacterial and viral infections.

The main strengths of our study is that we assessed drug-naïve FEP patients with a standardized antipsychotic treatment, namely risperidone. This is important to eliminate possible confounding factors when analyzing the effect of immunomodulation of antipsychotics. However, some limitations should be taken into account. The small number of patients may limit generalization of findings. Also, the analysis were made at baseline and after 10 weeks, and a longer period of follow-up

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

may give more information on the long-term effects of treatment on immune-inflammatory responses.

In conclusion, our findings indicate that drug-naïve FEP patients show a strong immune-inflammatory (IRS) (M1 + Th-1 + Th-17) response coupled with an anti-inflammatory response (CIRS), suggesting that monocytes, macrophages, Th-1, Th-17, Th-2 and Treg cells are induced. The findings indicate that FEP patients are prone to the detrimental effects of M1, Th-1, Th-17 and Th-2 cytokines, which could contribute to long-lasting abnormalities in brain circuitry. The findings support the hypothesis that, in FEP, the CIRS may contribute to recovery from the acute phase of illness. Enhancing the CIRS may be a new drug target in the treatment of FEP.

Acknowledgement

This study was supported by Fundação de Amparo à Pesquisado Estado de São Paulo (FAPESP 2010/08968-6; 2011/50740-5; 2014/07280-1), Brazil and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

Abbreviations: interferon-gamma(IFN- γ); interleukin 1beta (IL-1 β); tumor necrosis factor-alpha (TNF- α); interleukin(IL-6); soluble IL-6 receptor (sIL-6R); interleukin-2 (IL-2); soluble interleukin-2 receptor (sIL-2R); interleukin 4 (IL-4); interleukin-10 (IL-10); ,; IL-13: interleukin 13; IL-6: interleukin 6; IL-7: interleukin 7; IL-15: interleukin 15; IL-5: interleukin 5; IL-12: interleukin 12; IL-1ra: interleukin 1 receptor antagonist; IL-2: interleukin 2; IL-17: interleukin 17; IL-2R: interleukin 2 receptor; IL-4: interleukin 4; IL-8: interleukin 8; GM-CFS: granulocyte-macrophage

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

colony-stimulating factor; sTNF-R2: soluble tumor necrosis factor receptor 2; sTNF-R1: soluble tumor necrosis factor receptor1

M1: macrophage M1 profile;;Th1: T helper1 profile;Th2: T helper 2 profile;;

Treg: T regulatory profile; CIRS: Compensatory immune regulating reflex system;

IRS: immune-inflammatory response system (IRS); first episode psychosis (FEP); N-methyl-D-aspartate (NMDA)

References:

1. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B (2011) Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry 70 (7):663-671. doi:S0006-3223(11)00404-5 [pii] 10.1016/j.biopsych.2011.04.013

2. Anderson G, Berk M, Dodd S, Bechter K, Altamura AC, Dell'osso B, Kanba S, Monji A, Fatemi SH, Buckley P, Debnath M, Das UN, Meyer U, Muller N, Kanchanatawan B, Maes M (2013) Immuno-inflammatory, oxidative and nitrosative stress, and neuroprogressive pathways in the etiology, course and treatment of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 42:1-4. doi:10.1016/j.pnpbp.2012.10.008

3. Smith RS, Maes M (1995) The macrophage-T-lymphocyte theory of schizophrenia: additional evidence. Med Hypotheses 45 (2):135-141. doi:0306-9877(95)90062-4 [pii]

4. Goldsmith DR, Rapaport MH, Miller BJ (2016) A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. Mol Psychiatry 21 (12):1696-1709. doi:10.1038/mp.2016.3

5. Modabbernia A, Taslimi S, Brietzke E, Ashrafi M (2013) Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. Biol Psychiatry 74 (1):15-25. doi:10.1016/j.biopsych.2013.01.007

6. Maes M, Carvalho AF (2018) The Compensatory Immune-Regulatory Reflex System (CIRS) in Depression and Bipolar Disorder. Mol Neurobiol. doi:10.1007/s12035-018-1016-x

7. Maes M, Berk M, Goehler L, Song C, Anderson G, Galecki P, Leonard B (2012) Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. BMC Med 10:66. doi:10.1186/1741-7015-10-66 1741-7015-10-66 [pii]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

8. Upthegrove R, Manzanares-Teson N, Barnes NM (2014) Cytokine function in medication-naive first episode psychosis: A systematic review and meta-analysis. Schizophr Res 155 (1-3):101-108. doi:10.1016/j.schres.2014.03.005

9. Noto C, Ota VK, Santoro ML, Ortiz BB, Rizzo LB, Higuchi CH, Cordeiro Q, Belangero SI, Bressan RA, Gadelha A, Maes M, Brietzke E (2015) Effects of depression on the cytokine profile in drug naive first-episode psychosis. Schizophr Res 164 (1-3):53-58. doi:10.1016/j.schres.2015.01.026

10. Moudgil KD, Choubey D (2011) Cytokines in autoimmunity: role in induction, regulation, and treatment. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 31 (10):695-703. doi:10.1089/jir.2011.0065

11. Maes M, Bocchio Chiavetto L, Bignotti S, Battisa Tura G, Pioli R, Boin F, Kenis G, Bosmans E, de Jongh R, Lin A, Racagni G, Altamura CA (2000) Effects of atypical antipsychotics on the inflammatory response system in schizophrenic patients resistant to treatment with typical neuroleptics. Eur Neuropsychopharmacol 10 (2):119-124

12. Maes M, Meltzer HY, Bosmans E (1994) Immune-inflammatory markers in schizophrenia: comparison to normal controls and effects of clozapine. Acta Psychiatr Scand 89 (5):346-351

13. Zajkowska Z, Mondelli V (2014) First-episode psychosis: an inflammatory state? Neuroimmunomodulation 21 (2-3):102-108. doi:10.1159/000356536

14. Noto C, Ota VK, Gouvea ES, Rizzo LB, Spindola LM, Honda PH, Cordeiro Q, Belangero SI, Bressan RA, Gadelha A, Maes M, Brietzke E (2014) Effects of risperidone on cytokine profile in drug-naive first-episode psychosis. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 18 (4). doi:10.1093/ijnp/pyu042

15. Noto C, Ota VK, Gouvea ES, Rizzo LB, Spindola LM, Honda PH, Cordeiro Q, Belangero SI, Bressan RA, Gadelha A, Maes M, Brietzke E (2015) Effects of risperidone on cytokine profile in drug-naive first-episode psychosis. Int J Neuropsychopharmacol 18 (4). doi:10.1093/ijnp/pyu042

16. Capuzzi E, Bartoli F, Crocamo C, Clerici M, Carra G (2017) Acute variations of cytokine levels after antipsychotic treatment in drug-naive subjects with a first-episode psychosis: A meta-analysis. Neuroscience and biobehavioral reviews 77:122-128. doi:10.1016/j.neubiorev.2017.03.003

17. Kay SR, Fiszbein A, Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 13 (2):261-276

18. Leucht S, Davis JM, Engel RR, Kissling W, Kane JM (2009) Definitions of response and remission in schizophrenia: recommendations for their use and their presentation. Acta psychiatrica Scandinavica Supplementum (438):7-14. doi:10.1111/j.1600-0447.2008.01308.x

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

19. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological) 57 (1):289-300

20. Maes M, Bosmans E, Calabrese J, Smith R, Meltzer HY (1995) Interleukin-2 and interleukin-6 in schizophrenia and mania: effects of neuroleptics and mood stabilizers. J Psychiatr Res 29 (2):141-152

21. Maes M, Goossens F, Scharpe S, Calabrese J, Desnyder R, Meltzer HY (1995) Alterations in plasma prolyl endopeptidase activity in depression, mania, and schizophrenia: effects of antidepressants, mood stabilizers, and antipsychotic drugs. Psychiatry research 58 (3):217-225

22. Maes M, Delange J, Ranjan R, Meltzer HY, Desnyder R, Cooremans W, Scharpe S (1997) Acute phase proteins in schizophrenia, mania and major depression: modulation by psychotropic drugs. Psychiatry research 66 (1):1-11

23. Di Nicola M, Cattaneo A, Hepgul N, Di Forti M, Aitchison KJ, Janiri L, Murray RM, Dazzan P, Pariante CM, Mondelli V (2013) Serum and gene expression profile of cytokines in first-episode psychosis. Brain Behav Immun 31:90-95. doi:10.1016/j.bbi.2012.06.010

24. Schwarz MJ, Muller N, Riedel M, Ackenheil M (2001) The Th2-hypothesis of schizophrenia: a strategy to identify a subgroup of schizophrenia caused by immune mechanisms. Med Hypotheses 56 (4):483-486. doi:10.1054/mehy.2000.1203 S0306-9877(00)91203-9 [pii]

25. Degre M (1996) Interferons and other cytokines in bacterial infections. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 16 (6):417-426

26. Yusa T, Tateda K, Ohara A, Miyazaki S (2017) New possible biomarkers for diagnosis of infections and diagnostic distinction between bacterial and viral infections in children. J Infect Chemother 23 (2):96-100. doi:10.1016/j.jiac.2016.11.002

27. Degré M (1996) Cytokines and bacterial infections. Biotherapy 8 (3):219-228. doi:10.1007/bf01877208

28. Leonard B, Maes M (2012) Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. Neurosci Biobehav Rev 36 (2):764-785. doi:10.1016/j.neubiorev.2011.12.005 S0149-7634(11)00212-0 [pii]

29. Noto C, Ota VK, Santoro ML, Gouvea ES, Silva PN, Spindola LM, Cordeiro Q, Bressan RA, Gadelha A, Brietzke E, Belangero SI, Maes M (2015) Depression, Cytokine, and Cytokine by Treatment Interactions Modulate Gene Expression in Antipsychotic Naive First Episode Psychosis. Mol Neurobiol. doi:10.1007/s12035-015-9489-3

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

30. Connor TJ, Starr N, O'Sullivan JB, Harkin A (2008) Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma? Neurosci Lett 441 (1):29-34. doi:10.1016/j.neulet.2008.06.007

31. Kanchanatawan B, Hemrungrojn S, Thika S, Sirivichayakul S, Ruxrungtham K, Carvalho AF, Geffard M, Anderson G, Maes M (2018) Changes in Tryptophan Catabolite (TRYCAT) Pathway Patterning Are Associated with Mild Impairments in Declarative Memory in Schizophrenia and Deficits in Semantic and Episodic Memory Coupled with Increased False-Memory Creation in Deficit Schizophrenia. Molecular neurobiology 55 (6):5184-5201. doi:10.1007/s12035-017-0751-8

32. Lacey DC, Achuthan A, Fleetwood AJ, Dinh H, Roiniotis J, Scholz GM, Chang MW, Beckman SK, Cook AD, Hamilton JA (2012) Defining GM-CSF- and macrophage-CSF-dependent macrophage responses by in vitro models. Journal of immunology 188 (11):5752-5765. doi:10.4049/jimmunol.1103426

33. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, Fugger L (2008) Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol 172 (1):146-155. doi:10.2353/ajpath.2008.070690

34. Senior K (2009) Interleukin-17 and brain injury in stroke. Nature Reviews Neurology 5:524. doi:10.1038/nrneurol.2009.148

35. Willette AA, Coe CL, Birdsill AC, Bendlin BB, Colman RJ, Alexander AL, Allison DB, Weindruch RH, Johnson SC (2013) Interleukin-8 and interleukin-10, brain volume and microstructure, and the influence of calorie restriction in old rhesus macaques. Age (Dordr) 35 (6):2215-2227. doi:10.1007/s11357-013-9518-y

36. McLarnon JG (2016) Chemokine Interleukin-8 (IL-8) in Alzheimer's and Other Neurodegenerative Diseases. Journal of Alzheimers Disease & Parkinsonism 6 (6):1-4. doi:10.4172/2161-0460.1000273

37. Maes M, Song C, Yirmiya R (2012) Targeting IL-1 in depression. Expert opinion on therapeutic targets 16 (11):1097-1112. doi:10.1517/14728222.2012.718331

38. Gadani SP, Cronk JC, Norris GT, Kipnis J (2012) IL-4 in the brain: a cytokine to remember. J Immunol 189 (9):4213-4219. doi:10.4049/jimmunol.1202246

39. Mori S, Maher P, Conti B (2016) Neuroimmunology of the Interleukins 13 and 4. Brain Sci 6 (2). doi:10.3390/brainsci6020018

40. Liva SM, de Vellis J (2001) IL-5 induces proliferation and activation of microglia via an unknown receptor. Neurochem Res 26 (6):629-637

41. Simsek S, Yildirim V, Cim A, Kaya S (2016) Serum IL-4 and IL-10 Levels Correlate with the Symptoms of the Drug-Naive Adolescents with First Episode, Early Onset Schizophrenia. Journal of child and adolescent psychopharmacology 26 (8):721-726. doi:10.1089/cap.2015.0220

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 September 2018 doi:10.20944/preprints201809.0314.v1

42. Crow TJ (1981) Positive and negative schizophrenia symptoms and the role of dopamine. Br J Psychiatry 139:251-254

43. Altamura AC, Buoli M, Pozzoli S (2014) Role of immunological factors in the pathophysiology and diagnosis of bipolar disorder: comparison with schizophrenia. Psychiatry and clinical neurosciences 68 (1):21-36. doi:10.1111/pcn.12089

44. Maes M, Bosmans E, Ranjan R, Vandoolaeghe E, Meltzer HY, De Ley M, Berghmans R, Stans G, Desnyder R (1996) Lower plasma CC16, a natural anti-inflammatory protein, and increased plasma interleukin-1 receptor antagonist in schizophrenia: effects of antipsychotic drugs. Schizophr Res 21 (1):39-50

45. Sugino H, Futamura T, Mitsumoto Y, Maeda K, Marunaka Y (2009) Atypical antipsychotics suppress production of proinflammatory cytokines and up-regulate interleukin-10 in lipopolysaccharide-treated mice. Prog Neuropsychopharmacol Biol Psychiatry 33 (2):303-307. doi:10.1016/j.pnpbp.2008.12.006

46. Obuchowicz E, Bielecka-Wajdman AM, Paul-Samojedny M, Nowacka M (2017) Different influence of antipsychotics on the balance between pro- and anti-inflammatory cytokines depends on glia activation: An in vitro study. Cytokine 94:37-44. doi:10.1016/j.cyto.2017.04.004

47. da Cruz Jung IE, Machado AK, da Cruz IB, Barbisan F, Azzolin VF, Duarte T, Duarte MM, do Prado-Lima PA, Bochi GV, Scola G, Moresco RN (2016) Haloperidol and Risperidone at high concentrations activate an in vitro inflammatory response of RAW 264.7 macrophage cells by induction of apoptosis and modification of cytokine levels. Psychopharmacology (Berl) 233 (9):1715-1723. doi:10.1007/s00213-015-4079-7

48. Sarvari AK, Vereb Z, Uray IP, Fesus L, Balajthy Z (2014) Atypical antipsychotics induce both proinflammatory and adipogenic gene expression in human adipocytes in vitro. Biochemical and biophysical research communications 450 (4):1383-1389. doi:10.1016/j.bbrc.2014.07.005