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Peripheral immuno-inflammatory abnormalities in ultra-high risk of developing psychosis

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ABSTRACT

Background: Immuno-inflammatory imbalances have been documented in schizophrenia, but very little is known about the immunological changes prior to the onset of disease.
Objective: This work aimed to compare serum levels of pro- and anti-inflammatory cytokines in young subjects at ultra-high risk (UHR) of developing psychosis with age- and sex-matched healthy controls.
Methods: A total of 12 UHR and 16 age- and sex-matched healthy controls (HC) subjects were enrolled in this study. Clinical profile was assessed using the Comprehensive Assessment of At-Risk Mental States (CAARMS), Semi-Structured Clinical Interview for DSM-IV Axis-I (SCID-I) or Kiddie-SADS-Present and Lifetime Version (K-SADS-PL), and Global Assessment of Functioning (GAF) scale. Serum interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, IFN-γ, and IL-17 were measured by flow cytometry using the Th1/Th2/Th17 cytometric bead array.
Results: Compared with the healthy control group, patients in UHR showed increased IL-6 levels ($Z = -2.370$, $p = 0.018$) and decreased IL-17 levels in serum ($Z = -1.959$, $p = 0.050$). Levels of IL-17 positively correlated to the values in GAF symptoms (rho = 0.632, $p = 0.028$).
Conclusion: Our results suggest that immunological imbalances could be present in the early stages of psychosis, including in at-risk stages. Future studies should replicate and expand these results.

1. Introduction

Schizophrenia (SCZ) has been conceptualized as a neurodevelopmental disease in which the appearance of psychotic symptoms represents a relatively late manifestation (Lewis, 2002). In line with this conception, recent research effort has focused in identifying and prospectively evaluating individuals in pre- and subclinical stages (Phillips et al., 2000; Brietzke et al., 2011). Evidence indicates that 15% to 40% of individuals that fulfill criteria for ultra-high risk (UHR) develop a psychotic episode over a 2-year period (Correll, 2007). Although the phenomenology of early stages of psychosis has been increasingly more studied, it remains highly improbable that only clinical features alone could reliably identify who will and will not develop psychosis in follow-up (Mansur et al., 2012; Castro et al., 2015). Characterization of the biological underpinnings of UHR states could be an important step in disease model understanding, development of predictive biomarkers, and, subsequently, of preventive strategies (Perez et al., 2014; Labad et al., 2015; Zanini et al., 2015).

Accumulating evidence has indicated that the immune system is involved in the pathophysiology of psychosis. Cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) cross the blood–brain barrier (BBB) and modulate several molecular/cellular processes, including, but not limited to, monoamines metabolism (Miller, 2009). Immunological imbalances have already been identified in SCZ patients. A quantitative evaluation of 62 studies showed increased levels of the receptor antagonist of IL-1 (IL-1Ra) and soluble receptor of IL-2 (sIL-2R), as well as reduction in IL-6 and IL-2 (Potvin et al., 2008). Moreover, a recent meta-analysis of 40 studies, which considered the effects of clinical status, observed high levels of IL-1β, IL-6, and transforming growth factor-beta (TGF-β) during the acute phase of disease—an effect that was absent in the remission phase (Miller et al., 2011). In contrast,

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IL-12, interferon-gamma (IFN-γ), TNF-α, and soluble receptor for IL-2 (sIL-2R) were increased during acute psychosis as well remission (Miller et al., 2011). In addition, cytokine peripheral levels have also been relatively well studied in first episode psychosis (FEP), with studies demonstrating that immuno-inflammatory changes could be present in the early stage of disease (Brinholi et al., 2015; Noto et al., 2014; Upthegrove et al., 2014; Noto et al., 2015a, 2015b; Petrikis et al., 2015). A meta-analysis with all studies comparing individuals in FEP with healthy controls (HCs) found highly significant effect sizes for elevated IL-1, sIL-2r, IL-6, and TNF-α (Upthegrove et al., 2014). However, questions about the role of immune-inflammatory alterations in early stages of psychosis persist, specifically on the role of possible confounding factors, such as mood symptoms, comorbidities, and history of childhood maltreatment. For example, preliminary data from our group indicate that mood symptoms can influence cytokine levels in SCZ and FEP (Noto et al., 2011; Noto et al., 2015a, 2015b).

Moreover, relatively few studies assessed cytokines levels in at-risk states. Stojanovic et al. (2014) assessed serum IL-6, C-reactive protein (CRP), and plasmatic fibrinogen in a sample of 17 at-risk mental states (ARMS) subjects and verified that, compared with HCs, ARMS subjects had significantly increased levels of IL-6, which was also positively correlated with negative symptoms severity. A significant limitation of this pioneer study was the inclusion of only one cytokine, which precludes conclusions about a broader functioning of the immune system.

The aim of this study was to compare serum cytokine levels in a sample of subjects at UHR for developing psychosis with a healthy control group, as well as to verify if the impact of possible associated factors (e.g., mood symptoms, psychosocial functioning, body mass index, and history of childhood maltreatment) in cytokine levels.

2. Methods

2.1. Sample

Twelve UHR subjects within an age range from 14 to 26 years were recruited from the Recognition Program and Intervention in Risk Mental States (PRISMA Early Intervention Program), a specialized outpatient unit intended to treat and follow patients at ultra-high risk for developing psychosis settled in São Paulo, Brazil (Brietzke et al., 2011; Castro et al., 2015). We used the criteria for ultra-high risk for psychosis proposed and validated by Yung et al., according to the classification of the Comprehensive Assessment of At-risk Mental State (CAARMS) Scale (Yung et al., 2005). The criteria adopted for considering UHR can be seen in Table 1.

For the control group, healthy individuals were selected and matched for gender and age. They had no current or past history of mental disorders according to the Structured Clinical Interview for DSM-IV Axis 1 (SCID-1). In addition, we included only subjects with no history of use of psychotropic medications and no family history of a major psychiatric disorder (defined as unipolar depression, bipolar disorder [BD], suicide, or psychosis) in any first-degree relative.

The exclusion criteria for both groups were: (i) recent use of anti-inflammatories (up to and including the previous 2 weeks); (ii) long-term treatment with immunomodulatory drugs; and (iii) acute and chronic general medical conditions associated with imbalances in immune-inflammatory pathways such as infections, HIV infection, allergies, and autoimmune disorders (e.g., rheumatoid arthritis, inflammatory bowel disease). In addition, women of childbearing age were excluded if they were pregnant (or might be pregnant) or if they were in the postpartum period.

Clinical data were evaluated by the following scales: Montgomery-Åsberg Depression Rating Scale (MADRS), Young Mania Rating Scale (YMRS), Childhood Trauma Questionnaire (CTQ), Global Assessment of Functioning scale (GAF), and Global Assessment of Symptoms Scale (GAS).

The research protocol was approved by the Ethics in Research Committee and all participants above 18 years of age provided written informed consent prior to their enrolment in this study. For participants under 18 years old, the written informed consent was provided by the legal responder.

2.2. Blood collection

A total volume of 10 mL of peripheral blood was collected via arm venipuncture in BD SST II Advanced tubes (BD Biosciences). The collection of blood samples were carried out between 8 and 10 am after a minimum 12-hour fast. After collection, blood was allowed to clot for 30 min at room temperature and then centrifuged at 3200 rpm for 10 min to obtain serum. Serum was aliquoted and stored at −80 °C for a maximum time of 2 years.

2.3. Cytokine measurements

Cytokines (IL-2, IL-10, IL-4, IL-6, IFN-γ, TNF-α, and IL-17) were measured in duplicates and in a single experiment by mean of flow cytometry using the Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Kit (BD Biosciences). Acquisition was performed with a BD LSRFortessa™ 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., Pecs, Hungary). The detection limits for these assays were 2.6 (IL-2), 4.9 (IL-4), 2.4 (IL-6), 4.5 (IL-10), 3.8 (TNF), 3.7 (IFN-γ), and 18.9 pg/mL (IL-17). Values below detection limit were defined as zero. Percentage of total values above limit of detection for cytokines was 89.3% (IL-6), 82.1% (IL-17), and 64.3% (IFN-γ). Values for IL-2, TNF-α, IL-4, and IL-10 were above the detection limit in ~21.4% samples and, because of this, these cytokines were excluded from the analysis.

2.4. Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences, IBM SPSS 20.0 software (IBM Corporation, Armonk, NY). All variables were tested for normal distribution using the Shapiro-Wilk test. For continuous variables, differences between groups were analyzed with the Mann-Whitney *U* test; for categorical variables, the chi-squared test (χ^2) was used. Correlations between continuous variables were analyzed using Spearman's rank correlation test. Effect sizes for Mann-Whitney test were calculated as $r = Z/\sqrt{n}$ where Z was the standardized value of Mann-Whitney U test and n was the total sample size (Rosenthal and Rosnow, 1991). Level of significance was set at $\alpha < 0.05$ (two-tailed). The achieved power was calculated with the help of G*Power software version 3.1.6, using as settings

Table 1
Criteria for ARMS.

Ultra-high risk for psychosis
Attenuated positive symptoms Presence of positive symptoms in moderate severity but not reaching clearly the psychotic band; present more than one time per month for >1 h per week, in the past year, associated with a reduction in social and occupational functioning
Brief intermittent psychotic symptoms Presence of brief episodes of a full psychotic illness which might involve all of the symptoms of a psychosis (particularly delusions and hallucinations)
Trait and state risk factors Vulnerable family history of psychosis in a 1st-degree relative or a diagnosis of schizotypal personality disorder associated with a decline in social and occupational functioning

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