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Role of IL-6/RORC/IL-22 axis in driving Th17 pathway mediated immunopathogenesis of schizophrenia

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ABSTRACT

The immuno-inflammatory origin of schizophrenia in a subset of patients is viewed as a key element of an overarching etiological construct. Despite substantial research, the immune components exerting major effect are yet to be fully clarified. Disrupted T cell networks have consistently been linked to the pathogenesis of schizophrenia. Amongst the Th cell subsets, the Th17 cells have emerged as a paradigmatic lineage with significant functional implications in a vast number of immune mediated diseases including brain disorders such as schizophrenia.

The present study was aimed at examining the functional role of the Th17 pathway in schizophrenia. To address this, genotyping of IL17A (rs2275913; G197A) Single Nucleotide Polymorphism was carried out by the PCR-RFLP method in 221 schizophrenia patients and 223 healthy control subjects. Gene expression of two transcription factors STAT3 and RORC was quantified in a subset of drug naïve schizophrenia patients (n = 56) and healthy controls (n = 52) by TaqMan assay. The plasma levels of fifteen cytokines belonging to Th17 pathway were estimated in a subset of drug naïve schizophrenia patients (n = 61) and healthy controls (n = 50) by using Bio-Plex Pro Human Th17 cytokine assays.

The AA genotype was associated with higher total score of bizarre behaviour and apathy in female schizophrenia patients. A high gene expression level of RORC was observed in drug naïve schizophrenia patients. In addition, significantly elevated plasma levels of IL-6 and IL-22, and reduced levels of IL-1β and IL-17F were noted in schizophrenia patients. Taken together, these findings indicate a dysregulated Th17 pathway in schizophrenia patients.

1. Introduction

Although the precise mechanistic basis of schizophrenia remains enigmatic, the immuno-inflammatory theory of origin has gained prominence in recent years [1,2]. Data supporting this model have been obtained from peripheral blood, cerebrospinal fluid (CSF), post-mortem brain and neuroimaging studies, both in human as well as experimental animals [3–5]. Based on the evidence of a disrupted T-cell network, such as altered percentages of T cells and aberrant synthesis of cytokines by T cells, a pivotal role of T lymphocytes which are the producers of pro-inflammatory cytokines have been demonstrated as part of the immunopathogenetic pathways of schizophrenia [6–8]. T lymphocytes are primarily classified as T helper 1 (Th1) and T helper 2 (Th2) based on the type of cytokines they produce [9]. Th cells are further classified as T regulatory (Treg) cells, follicular T cells (Thf), and T helper 17 (Th17) cells. Amongst these, Th17 cells have emerged as a crucial lineage of Th cells given their pivotal roles in the immunopathogenetic pathways of human diseases with infectious, autoimmune and inflammatory origins [10]. Th17 cells are predominantly involved in mucosal defence of gut, skin and lungs, where interleukin (IL)-22 and IL-17A and IL-17F play key roles.

The differentiation, amplification, and stabilization of Th17 cells are distinct from other Th cell subsets, and are co-ordinated by action of

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multiple cytokines as well as transcription factors. The key cytokines involved in differentiation of Th17 cells are transforming growth factor beta (TGF-β), IL-6 and IL-1β, while cytokines involved in its amplification and stabilization include IL-21 and IL-23. In addition to this, two transcription factors, signal transducer and activator of transcription 3 (STAT3) and retinoic acid receptor-related orphan receptor (RORγt or RORC) are crucial in the early differentiation of Th17 cells. Th17 cells preferentially produce IL-17A, IL-17F, IL-21 and IL-22; these effector cytokines play a role in mucosal defence and mediate immune response predominantly against extracellular bacteria [11].

Emerging evidence both from human and animal studies suggest that pro-inflammatory cytokines produced by Th17 cells may promote autoimmune and immuno-inflammatory reactions in a large number of diseases with immunological underpinnings [11]. Th17 cells are also being increasingly implicated in central nervous system disorders owing their ability to cross the blood brain barrier (BBB), infiltrate the brain and induce neuroinflammation [12–14]. IL-17 and IL-22 receptors are expressed on BBB endothelial cells as well as gut mucosal cells [15]; ligand binding to these receptors leads to disruption of the BBB tight junction [16]. This process seems to help transmigration of Th17 cells across the BBB [16]. BBB dysfunction in schizophrenia influences neuronal and synaptic function as well as glutamate homoeostasis and is linked to impaired efficacy of antipsychotics and treatment resistance [17].

The importance of the Th17 pathway is envisaged in various neuropsychiatric disorders including schizophrenia, Autism spectrum disorders and depression, albeit supported by limited studies [18,19]. Recently, we proposed Th17 pathway related possible mechanisms that may underlie the immunopathogenesis of schizophrenia [20,21]. Commensurate with this, preliminary studies have demonstrated altered Th17 pathway elements in schizophrenia. In a recent study, an activated Th17 pathway accompanied by higher proportion of Th17 cells, elevated plasma levels of IL-17, IL-6 and interferon-gamma (IFN-γ) was demonstrated in drug naïve, first episode schizophrenia patients [19]. Further support towards a role of Th17 cells in the development of psychosis came from a study demonstrating an increased percentage of Th17 cells in adults with both psychotic symptoms and the 22q11.2 deletion syndrome. Th17 cell percentage was especially correlated to the presence of positive psychotic symptoms [22]. Another study showing significantly increased levels of growth-regulated oncogene (GRO), monocyte chemoattractant protein-1 (MCP-1), macrophage-derived chemokine (MDC) and soluble CD40 ligand (sCD40L) and decreased levels of IFN-γ, IL-2, IL-12p70 and IL-17 indicated a potential role of the IL-17 pathway in schizophrenia [23]. In addition, plasma levels of cytokines belonging to the Th17 pathway like IL-17, TGF-β1 and IL-23 were higher in patients with schizophrenia, and correlated with severity and aggressive behaviour [24].

Despite these advances, the precise role of the Th17 pathway is not adequately known. It is essential to determine the status of all the cytokines belonging to the Th17 pathway and the influence of transcription factors (STAT3 and RORC) on the Th17 pathway in schizophrenia. Further, examination of the effect of genetic variations within the IL17A gene on gene expression as well as plasma levels of IL-17 is necessary to understand genetic regulation of effector functions of Th17 cells. To address these issues, we adopted an integrated biochemical, genetic variation and gene expression study in drug naïve schizophrenia patients. The biochemical evaluation of Th17 pathway has been carried out by assessing the levels of fifteen cytokines belonging to the Th17 pathway.

2. Materials and methods

Schizophrenia patients (N = 221; age range: 18–45 years) attending the clinical services at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India and fulfilling Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria were considered for this study. Amongst these, a subset of patients (n = 61) who were either drug-naïve (i.e., never treated with any psychotropic medications including antipsychotics) or drug-free (i.e. not having been treated with oral medication for at least 6 weeks or with depot antipsychotics for at least 3 months) were considered for gene expression and cytokine estimation studies. Diagnosis was established using the Mini International Neuropsychiatric Interview (MINI) Plus [25], which was further assessed and confirmed independently by a qualified psychiatrist. The assessment of demographic and clinical characteristics (history of presenting illness as well as any other medical illness, family & personal history as well as antipsychotic-naïve/antipsychotic-free status) of the patients were ascertained with reliable inputs from first-degree relatives.

The scale for Assessment of Positive Symptoms (SAPS) [26], a 34-item tool which scores symptom severity under four domains- hallucinations, delusions, bizarre behaviour and formal thought disorder on a 6-point Likert-type scale, and the Scale for Assessment of Negative Symptoms (SANS) [27], a 25-item tool which scores symptom severity under six domains-affective flattening or blunting, alogia, avolition-apathy, anhedonia-asociality and attention on a 6-point Likert-type scale were used to assess clinical symptoms. These scales were administered with good inter-rater reliability.

A total of 223 healthy controls were recruited after ruling out any psychiatric diagnosis using the MINI plus as well as a comprehensive mental status examination. Amongst these, a subset of healthy controls (n = 52) matched for age- and gender were considered for gene expression and cytokine estimation studies. None of the controls had family history of psychiatric disorder in first-degree relatives. The subjects (patients/controls) did not have recent history of high grade fever/infection within the past six weeks or any co-morbid medical disease that could potentially influence the immune system; none had co-morbid substance abuse or dependence. The study was conducted after obtaining approval from the institutional ethics committee as well as written informed consent from the patients and healthy volunteers.

2.1. Sample collection

From all the consenting participants, 10 mL peripheral blood was drawn from the median cubital vein into Ethylenediaminetetraacetic acid (EDTA)-coated vacutainers under aseptic conditions. The plasma and buffy coat were separated from whole blood by centrifugation and stored at −80 °C deep freezer. The plasma underwent only one freeze thaw cycle. The genomic DNA was extracted from buffy coat using commercial spin column method (Qiagen, Inc, Limburg, Netherlands). The total RNA was extracted by using commercial spin column method (Qiagen, Inc, Limburg, Netherlands) from peripheral blood mononuclear cells (PBMCs).

2.2. Genotyping

The G197A Single Nucleotide Polymorphism (SNP) of IL17A is located within the binding motif of the nuclear factor activated T cells (NF-AT), which is a critical regulator of IL17 A promoter activity. Importantly, G197A SNP was shown to be associated with production of IL-17A, and this suggests a functional role of this SNP in the regulation of IL-17A [28]. Genotyping of IL17A (rs2275913; G197A) SNP was carried by PCR-restriction fragment length polymorphism (PCR-RFLP) method in 221 schizophrenia patients and 223 healthy subjects. The PCR amplification was carried out in Eppendorf Nexus Gradient Mastercycler (Eppendorf, Germany). For PCR amplification, 10 μL reaction mix containing 5 μL of EmeraldAmp GT PCR Master mix (2X Premix), 0.2 μL of each forward and reverse primers (10 μM), 3 μL of RNase free water and 1.6 μL of genomic DNA (10 ng) was used. The forward and reverse primer sequences are 5′-GCATAACTCTTCTGGCAGCTGTA-3′; 5′-GTATTTCTGGACCGTGGGCA-3′, respectively. The amplification was carried out at 94 °C for 5 min followed by 35 cycles of 40 s at 94 °C for denaturation, 30 s at 59 °C for annealing, 1 min at 72 °C for extension, with a final extension at 72 °C for 10 min. An overnight digestion with 5 units XmnI at 37 °C yielded following bands: GG - 445 bp , GA-445 + 292 + 153 bp, AA-292 + 153 bp in a 3% agarose gel. The gel