Review Article

Methodological Issues in Cytokine Measurement in Schizophrenia

Maju Mathew Koola

ABSTRACT

There is mounting evidence that inflammation is a major factor in the pathophysiology of schizophrenia. Inflammatory status is commonly ascertained by measuring peripheral cytokine concentrations. An issue concerning research on inflammation and schizophrenia relates to assay methodology. Enzyme-linked immunosorbent assay (ELISA) is the most widely used and the gold standard method used to measure cytokine concentrations. ELISA has a number of limitations. Both ELISA and multiplex are limited by not being able to distinguish between bioactive and inactive molecules and the matrix and heterophilic (auto-) antibody interference. Multiplex assays when combined with gene expression analysis and flow cytometry techniques such as fluorescence-activated cell sorting may be useful to detect abnormalities in specific immune pathways. Peripheral blood mononuclear cells cultures, to evaluate *in vitro* lipopolysaccharide-induced cytokine production, may be a better technology than measuring cytokines in the serum. The purpose of this paper is to shed light on major methodological issues that need to be addressed in order to advance the study of cytokines in schizophrenia. We make a few recommendations on how to address these issues.

Key words: *Cytokines, flow cytometry, peripheral blood mononuclear cells culture, schizophrenia*

INTRODUCTION

Cytokines are produced by many types of cells including immune cells (e.g., macrophages, lymphocytes, and mast cells), endothelial cells, fibroblasts, and stromal cells. Current cytokine assays are designed to measure cross-sectional, peripheral cytokine concentrations, regardless of the source of the cytokine(s). Many cytokines have an opposing and sometimes complimentary effect to each other; thereby, modulating the severity of the immune response. There is a growing body of evidence of inflammation pathophysiology in schizophrenia.[1-3]

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The purpose of this review paper was to shed light on limitations and major methodological issues and how to address them in order to advance the study of cytokines in schizophrenia.

CYTOKINE MEASUREMENT ISSUES

Enzyme-linked immunosorbent assay (ELISA) is the most widely used and the gold standard method used to measure cytokine concentrations. ELISA has a number of limitations: Binding affinity of

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Department of Psychiatry and Behavioral Sciences, George Washington University School of Medicine and Health Sciences, Washington, DC, USA

Address for correspondence: Dr. Maju Mathew Koola
Clinical Research Program, Sheppard Pratt Health System, 6501 N Charles St., Baltimore, MD 21204, USA. E-mail: majujuju@yahoo.com

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antibodies can vary, the method requires large sample volume, it has a narrow dynamic range, can measure only one protein at a time, and is costly. Multiplex immunoassays can measure multiple cytokines simultaneously, and there are no specific limitations to the multiplex assays other than the two mentioned in the next sentence. Both ELISA and multiplex are limited by not being able to distinguish between bioactive and inactive molecules and the matrix and heterophilic (auto-) antibody interference. These antibodies cause false positive and false negative signals by binding to either the capture antibody, detection antibody or to the antigen.[4-5]

Most of the studies included in the meta-analyses,[1,2] used ELISA, but there were a few exceptions. Out of the 40 studies (in supplement),[2] 35 studies used ELISA, one study each used semimicroassay,[6] radioimmunoassay (RIA),[7] bioassay,[8] sandwich,[9] and cytometric bead array.[10] ELISA, RIA, and bioassay were used in 83%, 9%, and 8% of the studies, respectively (supplement).[1] Several studies used peripheral blood mononuclear cells (PBMC) cultures stimulated with lipopolysaccharide (LPS) or phytohemagglutinin.[11-16] RIA was used by a few studies.[17-19] Two studies used stimulated T cell culture.[20,21] In three studies, within the same study, interleukin 2 (IL-2) was measured by RIA and IL-6 and IL-8 were measured by ELISA.[19,22,23] One study did bioassay using the IL-6 dependent hybridoma cell line B9.[8] In none of the studies were any data presented to compare the similarity of results across different assays, nor were any data presented to suggest which cytokine measurement tests produce the most reliable and consistent results.

THE CHICKEN AND THE EGG CONUNDRUM

Another largely unaddressed question regarding the association between cytokines and schizophrenia is whether observed cytokine changes in schizophrenia are part of the causal pathway leading to the disease, or a secondary consequence of treatment, e.g., weight gain or result from lifestyle concomitants in people with schizophrenia, such as smoking. The issue of secondary consequence of treatment has been addressed in the Miller *et al*. meta-analysis.[2] The first episode participants were drug naïve, suggesting that cytokine abnormalities in schizophrenia are independent of antipsychotic medications.[2] A further possibility is that cytokines may be elevated as a consequence of the hypothalamic-pituitary-adrenal axis arousal induced by distressing psychiatric symptoms.

HOW TO DESIGN FUTURE STUDIES

Careful attention should be paid to the experimental design, sample collection, preparation, and storage; all these may have an impact on the study results.[5,8,24-28] As previously reported,[29] future studies should validate the cell sources (lymphocytes, monocytes, etc.) of specific cytokines and the complex interactions among cytokines and its association with oxidative stress and other systems. Although peripheral cytokine concentrations are easier to measure and have been mostly used in studies of schizophrenia, more research is needed to examine the association between cerebrospinal fluid and blood concentrations. Although the meta-analysis[2] examined the longitudinal changes in cytokines, antipsychotic treatment was not standardized. Future studies are warranted to validate the findings of longitudinal changes in cytokines; this evidence is scant in the literature.

Multiplex assays when combined with gene expression analysis and flow cytometry techniques such as fluorescence-activated cell sorting (FACS) may be useful to detect abnormalities in specific immune pathways.[30] PBMC cultures, to evaluate *in vitro* LPS-induced cytokine production, may be a better technology than measuring cytokines in the serum.[31-36] Cytokines in serum may be vulnerable to differential concentrations between the site of cytokine release and blood concentrations and the relatively short half-life of cytokines. The use of PBMC cultures complements the collection of peripheral cytokine measures. Significant increases in peroxisome proliferator-activated receptor gamma, sterol regulatory element-binding transcription factor 1, IL-6 and tumor necrosis factor alpha, and decreases in peroxisome proliferator-activated receptor alpha and C/enhancer-binding protein alpha and mRNA levels using stimulated PBMC were found in 31 participants with schizophrenia compared to 31 controls.[37] Describing FACS and PBMC culturing in detail is beyond the scope of this paper. Investigators should decide, after consulting with a psychoneuroimmunologist, which tests to be done.

CONCLUSIONS AND FUTURE DIRECTIONS

In sum, there is considerable evidence for a role of inflammation in the pathophysiology of schizophrenia. The use of standardized methodology for cytokine measurement will enhance the replicability of results across studies. This will advance the field for potential integration of pathophysiology with novel therapeutic discovery targeting inflammatory mechanisms.

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Conflicts of interest

There are no conflicts of interest.

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