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Full-length Article

Peripheral inflammatory cytokines and immune balance in Generalised Anxiety Disorder: Case-controlled study

Ruihua Hou [a,*], Matthew Garner [a,b], Clive Holmes [a], Clive Osmond [c], Jessica Teeling [d], Laurie Lau [a], David S. Baldwin [a,e]

[a] Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, United Kingdom
[b] Department of Psychology, University of Southampton, United Kingdom
[c] Medical Research Council Lifecourse Epidemiology Unit, University of Southampton, United Kingdom
[d] Biological Sciences, University of Southampton, United Kingdom
[e] University Department of Psychiatry and Mental Health, University of Cape Town, South Africa

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ABSTRACT

Introduction: Previous investigations have demonstrated that major depression is associated with particular patterns of cytokine signalling. The primary aim of this study was to examine peripheral pro-inflammatory and anti-inflammatory cytokines and immune balance in Generalised Anxiety Disorder (GAD). *Methods:* A case-controlled cross-sectional study design was employed: 54 patients with GAD and 64 healthy controls were recruited. Participants completed self-report measures of anxiety and depression. Two pro-inflammatory and two anti-inflammatory cytokines were measured using multiplex technology. *Results:* Case-control logistic regression analyses revealed significant differences in serum levels of IL-10, TNF-α, and IFN-γ between GAD and control groups after adjusting for age, gender, body mass index, smoking and alcohol consumption: these group differences were independent of the presence or degree of depression. Comparison of pro-inflammatory to anti-inflammatory cytokine ratios indicated that there were significantly higher ratios of TNF-α/IL10, TNF-α/IL4, IFN-γ/IL10, and IFN-γ/IL4 in the GAD group compared to the control group. *Conclusions:* This study is the first to investigate both pro- and anti-inflammatory cytokines and their balance in patients with GAD in comparison to healthy controls. The findings indicate a relatively increased pro-inflammatory response and decreased anti-inflammatory response and provide the first demonstration of an altered cytokine balance in GAD. Serum cytokine levels in GAD were independent of the presence of depression.

1. Introduction

Research into psychoneuroimmunology (PNI) has led to substantial advances in understanding of the reciprocal interactions between the central nervous system and the immune system in neuropsychiatric disorders (Ader and Cohen, 1975; Ader et al., 1995; Miller et al., 2009; Raison et al., 2006; Leonard and Myint, 2009). Evidence from experimental and clinical research shows the pivotal roles of *cytokine* signalling to the brain to produce neurochemical, neuroendocrine, neuroimmune, and behavioural changes (Kronfol and Remick, 2000; Maier, 2003; Loftis et al., 2010; Capuron and Miller, 2011; Dantzer et al., 2008; Müller and Schwarz, 2007). The presence of inflammatory responses, and in particular, the role of cytokines in major depression has been addressed in numerous studies. However, neuroinflammatory markers in anxiety disorders have been studied less extensively. There is a need for better understanding of both the heterogeneous role of specific cytokines and immune balance in anxious states and in different anxiety disorders (Hou et al., 2013).

Cytokines are soluble bioactive mediators released by various cell types both at the periphery (such as monocytes and macrophages) and in the brain (such as microglia, astrocytes, and endothelial cells), which operate within a complex network and can act synergistically or antagonistically. Based on the functional profile of an immune response, cytokine production is broadly orchestrated by T helper 1 cells (Th1) which generally mediate a pro-inflammatory cellular immune response, and T helper 2 cells (Th2) which enhance anti-inflammatory and humoral immune reactions. The *pro-inflammatory cytokines,* tumour necrosis

* Corresponding author. at: University Department of Psychiatry, Clinical and Experimental Sciences, University of Southampton Faculty of Medicine, Academic Centre, College Keep, 4-12 Terminus Terrace, Southampton SO14 3DT, United Kingdom.
E-mail address: r.hou@soton.ac.uk (R. Hou).

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factor-alpha (TNF-α) and interferon-γ (IFN-γ), prime a Th1 response, and enhance the elimination of intracellular pathogens, while the *anti-inflammatory cytokines*, interleukin (IL)-4 and IL-10, enhance a Th2 response, enabling phagocytosis of extracellular pathogens, tissue repair and dampening the synthesis of pro-inflammatory cytokines (Kronfol and Remick, 2000). The balance between Th1 and Th2 cytokines is an important determinant in regulating the inflammatory response, and a delicate balance of pro- and anti-inflammatory cytokines is required for normal neuropsychological functioning (Loftis et al., 2010; Dantzer et al., 2008).

Since signs of immune disturbances in depression were first reported in early 1990s (Maes et al., 1990, 1991, 1992a,b) the presence of inflammatory responses and the role of cytokines in major depression have been extensively studied. The high comorbidity of anxiety disorders and major depression and similar effects of antidepressants suggest common neurobiological substrates. In addition, the pronounced response of central and peripheral cytokines to stress has prompted further interest in the role of cytokines in the pathogenesis of anxiety disorders.

Generalised Anxiety Disorder (GAD), the most common impairing anxiety disorder, is a common and frequently chronic condition characterized by excessive, uncontrollable and often irrational worry about everyday things. GAD affects approximately 1.9–5.1% of the general population, and 8% of patients in primary care (Wittchen et al., 2011). Impairments of GAD are similar in magnitude to those of major depression (Hoffman et al., 2008). Camacho (Camacho, 2013) proposed that anxious-depression should be considered as a chronic inflammatory phenomenon but the only longitudinal study found the association between GAD and increased C-reactive protein (CRP) level to be attributable to body mass index (BMI) and medication use (Copeland et al., 2012). A large cohort study examined the association between anxiety disorders (including GAD, social phobia, PD, and agoraphobia) and inflammation (Vogelzangs et al., 2013 Apr 23), and the authors reported elevated CRP levels in male patients with current anxiety disorders and immune dysregulation in patients with a late-onset anxiety disorder. In addition, an integrated specificity model emphasizes specific patterns of biological responses to specific psychological states (Kemeny, 2003; Moons et al., 2010) and an anxiety-specific effect on inflammatory activity in clinically anxious individuals has been reported (O'Donovan et al., 2010). It is uncertain whether anxiety is associated with inflammatory activity in GAD either through a specific anxiety pathway or through a more general negative affective pathway.

The primary aims of our study, therefore, were to (Ader and Cohen, 1975) examine pro- and anti- inflammatory cytokine levels and ratios in patients with GAD in comparison to healthy controls; and (Ader et al., 1995) determine whether peripheral inflammatory cytokine levels in GAD are independent of depression. The main predictions were as follows: Hypothesis 1: There will be differences of serum cytokine levels as well as pro-inflammatory to anti-inflammatory cytokine ratios between the GAD group and the control group; Hypothesis 2: The effect of GAD on cytokine levels will be independent of depression.

2. Methods

2.1. Participants

A total of 54 patients (aged between 18 and 65 years with BMI between 18.5 and 29.9) with a primary diagnosis of GAD were recruited from community mental health team outpatient clinics and general practice surgeries. All patients met DSM-IV and ICD-10 diagnostic criteria for GAD. All patients completed a pre-test screening interview comprising a structured diagnostic Mini International Neuropsychiatric Interview – MINI (Sheehan et al., 1998) and the 7-item Generalised Anxiety Disorder Questionnaire (GAD-7) with a threshold score of 10 points (Spitzer et al., 2006). Due to high comorbidity of anxiety and depression in GAD, and in order to explore the influence of depression, patients with coexisting depressive symptoms were not excluded. 64 healthy controls were recruited from the community by advertising on posters and internet during the same period. All participants were able to understand both spoken and written English. Participants were excluded if they reported intake of any medication with known immune-modulating effects (such as glucocorticoids), had acute or chronic organic illnesses, or met criteria for additional mental disorders. Participants who had experienced any inflammatory event within the 2 weeks before the assessment were excluded. Patients taking anxiolytic drugs were not excluded.

The study was approved by the National Research Ethics Service Committee Health Research Authority South Central – Portsmouth (Reference Number 11/SC/0484).

2.2. Self-reported questionnaire measures

The following self-reported questionnaire measures were used:

1) The Hospital Anxiety Depression Scale (HADS), a well-validated measure of depression and anxiety, which consists of two 7-item subscales (Zigmond and Snaith, 1983).
2) The Perceived Stress Scale (PSS), a well-validated and widely used measure of subjective stress (Cohen et al., 1983). Participants rated the degree to which they perceived their lives to be unpredictable, uncontrollable, and overwhelming. Total scores range from 0 to 40, with higher scores indicating greater stress.
3) The Anxiety Sensitivity Index (ASI), a self-report measure of fears of arousal-reactive bodily symptoms (Blais et al., 2001), which is extensively used in clinical and health psychology research and has acceptable psychometric properties.

A structured general information questionnaire determining the socio-demographic and clinical features of participants was also employed.

2.3. Measure of peripheral inflammatory cytokines

A sample of 10 ml venous blood was taken from all participants at the same time of day (9:00–10:00AM) and centrifuged for 15 min at 2500 rpm. The cell free-serum was collected and aliquoted in freezer vials and stored at −80° C until further analysis. Serum levels of two pro-inflammatory cytokines (TNF-α and IFN-γ) and two anti-inflammatory cytokines (IL-4 and IL-10) were measured using a multiplex ultra-sensitive immunoassay – Meso Scale Discovery (MSD, USA). Calibrators were run in duplicate to generate a stand curve which was modelled using least squares fitting algorithms so that signals from samples with known levels of the analyte can be used to calculate the concentration of analyte in the sample. The sensitivity was indicated by the lower limit of detection (LLOD) of these cytokines (IL-4: 0.31 pg/ml, IL-10: 0.36 pg/ml, TNF-α: 0.48 pg/ml and IFN-γ: 0.39 pg/ml). Processing of blood samples was based on a protocol provided for human multiplex assays and recommendations for clinical trials (de Jager et al., 2009).

2.4. Study design and procedure

A case-controlled cross-sectional cohort design was employed. All potential participants were given detailed information sheets

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