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Full-length Article

TNFAIP3, a negative regulator of the TLR signaling pathway, is a potential predictive biomarker of response to antidepressant treatment in major depressive disorder

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ABSTRACT

Inflammation and abnormalities in Toll-like receptor (TLR) expression and activation have been linked to major depressive disorder (MDD). However, negative regulators of TLR pathways have not been previously investigated in this context. Here, we sought to investigate the association of depression severity, measured by the 17-item Hamilton Depression Rating Scale (HAMD-17), with mRNA expression levels of negative regulators of the TLR pathway, including SOCS1, TOLLIP, SIGIRR, MyD88s, NOD2 and TNFAIP3, in peripheral blood mononuclear cells (PBMCs) from 100 patients with MDD and 53 healthy controls, before and after treatment with antidepressants. Positive regulators of the TLR4 pathway, including Pellino 1, TRAF6 and IRAK1, were also investigated. Among all patients, MyD88s, and TNFAIP3 mRNAs were expressed at lower levels in PBMCs from patients with MDD. Multiple linear regression analyses revealed that TNFAIP3 mRNA expression before treatment was inversely correlated with severity of depression and effectively predicted improvement in HAMD-17 scores. Among 79 treatment-completers, only TNFAIP3 mRNA was significantly increased by treatment with antidepressants for 4 weeks. Treatment of human monocytes (THP-1) and mouse microglia (SIM-A9) cell lines with fluoxetine significantly increased TNFAIP3 mRNA expression and suppressed IL-6 levels. The suppressive effect of fluoxetine on IL-6 was attenuated by knockdown of TNFAIP3 expression. These findings suggest that both dysfunction of the negative regulatory system in patients with MDD and antidepressant treatment exert anti-inflammatory effects, at least in part through increased expression of the *TNFAIP3* gene. They also indicate that modulating expression of the *TNFAIP3* gene to rebalance TLR-mediated inflammatory signaling may be potential therapeutic strategy for treating MDD.

1. Introduction

Major depressive disorder (MDD) represents a combination of mood, anxiety, cognition, sleep, and appetite symptoms that last for more than 2 weeks. MDD, which is responsible for 7.4% of total disability-adjusted life years (DALYs) worldwide (Whiteford et al., 2013), is highly associated with inflammation (Maes et al., 2015; Miller and Raison, 2016; Vogelzangs et al., 2014). One meta-analysis reported elevated levels of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-6, in patents with MDD (Dowlati et al., 2010). Studies have also noted that MDD is associated with increased monocyte numbers (Seidel et al., 1996) and prostaglandin E2 secretion (Nishino et al., 1989). The currently favored hypothesis is that pro-inflammatory cytokines penetrate into the central nervous system and alter the activity of indoleamine 2,3-dioxygenase (IDO), which activates the kynurenine pathway and decreases the level of serotonin (Haroon et al., 2012; Myint and Kim, 2014).

It has been reported that treatments for depression are associated with a reduction in inflammation. Selective serotonin reuptake inhibitors (SSRIs) have been shown to diminish the output of interferon-γ (IFN-γ) in whole blood stimulated with

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lipopolysaccharide (LPS) (Maes et al., 1999). Fluoxetine, an antidepressant of the SSRI class, have been shown to suppress LPS-stimulated expression of IFN-γ–inducible protein 10, also known as C-X-C motif chemokine ligand 10 (CXCL10), in human monocytes (Tsai et al., 2014). Fluoxetine was also demonstrated to reduce LPS-induced pro-inflammatory IL-6 and TNF-α in human peripheral blood mononuclear cells (PBMCs) (Waiskopf et al., 2014). A variety of pathways, including the 5-HTT (5-hydroxytryptamine transporter), nuclear factor-kappB (NF-κB), IL-10, and cAMP have been investigated for their role in mediating the actions of SSRIs (Walker, 2013). Fluoxetine was also shown to inhibit LPS-induced decreases in intracellular acetylcholinesterase (AChE-S), which interacts with NF-κB–activating intracellular RACK1 (receptor for activated C kinase 1) (Waiskopf et al., 2014). However, the specific molecular mechanisms by which antidepressants of the SSRI class reduce inflammation have not yet been fully elucidated.

In terms of innate immune responses to MDD, recent studies have demonstrated an association between activation of Toll-like receptor (TLR)-4–mediated signaling and depression (Henry et al., 2016; Hung et al., 2014). Clinical studies have shown that TLR4 expression in the prefrontal cortex is enhanced in patients with MDD (Garate et al., 2014). Altered peripheral expression of TLRs appears to be associated with a heightened inflammatory state and depression (Crupi and Cuzzocrea, 2016). In addition, clinical studies have reported up-regulated TLR4 signaling in PBMCs in patients with MDD (Hung et al., 2014). Notably, TLR4 was found to be an independent risk factor for MDD severity and shown to be associated with symptoms of depression, including body weight loss and anxiety (Breese et al., 2008; Wu et al., 2015). We recently reported that antidepressant treatment attenuated increases in TLR4 mRNA associated with MDD (Hung et al., 2016), suggesting a possible interaction between antidepressants and TLR4 signaling pathways through an as yet unclear molecular mechanism(s).

Negative regulators of TLR4 signaling have been shown to intersect almost every step of the TLR signaling pathway, serving to protect against the potential harm of a prolonged, TLR-induced cytokine storm by controlling the magnitude of the peak response and/or duration of the response. IRAK3 (IL1 receptor-associated kinase 3), SOCS1 (suppressor of cytokine signaling 1), MyD88s (myeloid differentiation 88 short), TOLLIP (Toll-interacting protein), TNFAIP3 (TNF α-induced protein 3), ST2L (suppressor of tumorigenicity 2, full-length form), and SIGIRR (single immunoglobulin IL-1R–related receptor) are among the negative regulators of TLR-mediated immune responses (Liew et al., 2005). One of these important negative regulators, TNFAIP3 (also known as A20), acts as a deubiquitinase with specificity for lysine 63 (K63)-linked ubiquitin chains on TRAF6 (TNF receptor-associated factor 6) to suppress NF-κB activation and inflammatory responses (Shembade and Harhaj, 2012). While TNFAIP3 expression was reported to be associated with bipolar disorder (Barzman et al., 2014; Padmos et al., 2008), whether regulation of this negative regulator of TLR-mediated signaling occurred in MDD patients treated with antidepressants remains unknown.

Here, we investigated differences in the gene expression profile of positive and negative regulators of TLR signaling in patients with MDD and sought to determine possible anti-inflammatory mechanisms underlying the actions of the antidepressant, fluoxetine.

2. Materials and methods

2.1. Experimental design

Inpatients with MDD were recruited from the psychiatric ward of Kaohsiung Chang Gung Memorial Hospital, Taiwan, from August 2013 to May 2016. Blood samples for mRNA analysis were obtained from patients before and after antidepressant treatment, and from 53 healthy controls at baseline. Institutional Review Board approval was obtained from the hospital ethics committee (101-5012A3, 103-5114B and 103-6984A3). After receiving verbal and written information about the study, patients and healthy controls provided written consent to participate.

2.2. Participants

Patients with MDD were screened by two psychiatrists before entering the study. The screening steps, which were similar to those described in our previous work (Hung et al., 2016), included a Structured Clinical Interview for DSM-IV Axis I Disorders as well as a detailed assessment of current psychiatric symptoms and previous treatment. The 17-item Hamilton Depression Rating Scale (HAMD-17) was used by the same psychiatrists to assess the severity of depression. Patients with psychotic disorders, substance dependence (including alcohol), severe metabolic syndrome, severe obesity (body mass index [BMI] > 34 kg/m^2) or systemic inflammatory disease, or those who received antibiotics, anti-inflammatory or immune-modulating drugs, were excluded from the study. All patients were tested for blood pressure and received chest X-rays, electrocardiographic examinations, and routine blood tests after hospitalization to exclude possible chronic systemic physical illness. Enrolled patients reported no antidepressant use for at least 1 week before entering the study. Healthy controls, recruited from the community, had neither a personal history nor a first-degree relative with a psychiatric disorder. The same psychiatrist who performed screens of MDD patients assessed the healthy control group using Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) criteria to rule out psychiatric disease.

After a clinical examination, blood samples were taken at two time points: at inclusion (baseline) and 4 weeks after initiating antidepressant treatment. Between these two time points, patients were hospitalized in the psychiatric ward of Kaohsiung Chang Gung Memorial Hospital with good drug adherence, regular sleep-wake cycles, a well-controlled diet, and limited smoking.

2.3. Treatment

Treatment was administered as dictated by medical considerations, meaning that the choice of treatment was not influenced by the study and was chosen based on clinical judgment. After screening at baseline, chosen antidepressants were administered and recorded. The antidepressants included escitalopram (10–20 mg/d; n = 14), fluoxetine (40–80 mg/d; n = 12), paroxetine (20–40 mg/d; n = 12), sertraline (75 mg/d; n = 1) duloxetine (60–120 mg/d; n = 25), venlafaxine (37.5–225 mg/d; n = 7), bupropion (150–300 mg/d; n = 3), and agomelatine (25–50 mg/d; n = 5). All patients were administered benzodiazepines as anxiolytics or hypnotics, and had not received their medication for at least 8 h prior to blood sampling. Supportive psychotherapy sessions were provided one to two times, and regular activities were suggested during hospitalization.

2.4. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

Venous blood (5 mL) samples were drawn between 8:00 am and 10:00 am, after patients had fasted for 9 h. PBMCs were isolated from venous blood samples by Ficoll-Paque (GE, #17-5442-02) density gradient centrifugation. The isolated PBMCs were labeled with BD IMag anti-human CD14 Magnetic Particles – DM (BD Biosciences, #557769) according to the Magnetic Labeling Protocol. The labeled fraction containing CD14(+) monocytes from 24 completers was collected for further analysis. The tubes were