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Association between toll-like receptors expression and major depressive disorder

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ABSTRACT

Accumulating evidences suggest that Toll-like receptors (TLRs) were involved in the pathophysiology of major depressive disorder. TLR4 was thought to be associated with major depressive disorder in animal model, but the others were still unknown. In order to examine TLR1-9 mRNA expression levels in peripheral blood and their relationships with the psychopathology of major depressive disorder, 30 patients with major depressive disorder were compared with 29 healthy controls. The 17-item Hamilton Depression Rating Scale (HAMD-17) was used to assess the severity of major depression. The mRNA expression levels of TLRs were examined in parallel with a housekeeping gene using real-time polymerase chain reaction (RT-PCR). Analysis of covariance with age and body mass index adjustment revealed a significantly higher expression of TLR3, 4, 5 and 7 mRNA but lower expression of TLR1 and 6 in patients with major depressive disorder as compared with healthy controls. Multiple linear regression analysis revealed that TLR4 was an independent risk factor relating to severity of major depression. These findings suggest that TLRs, especially TLR4, may be involved in the psychopathology of major depression.

1. Introduction

Toll-like receptors (TLRs) are pattern recognition receptors proposed as main agents of the innate immune response, constituting the first line of defense against invading microorganisms (Akira and Takeda, 2004). TLRs recognize endogenous ligands, termed as damage-associated molecular patterns, and mediate an inflammatory response in the host to injury and stress (Piccinini and Midwood, 2010).

There are various types of TLRs, and each have different cell-surface receptors; these include: TLR4, which recognizes lipopolysaccharide (LPS) present in Gram-negative bacteria; TLR2 and its co-receptors TLR1/TLR6, which sense bacterial lipoproteins and lipoteichoic acid; and TLR5 that recognizes bacterial flagellin. Another subtype of receptors are localized in the endosomal cell compartment and include TLR3, which recognizes double-stranded (ds) RNA; TLR9, which senses unmethylated CpG motifs present in viral and bacterial genome and parasites; and TLR7, which binds single-stranded (ss) DNA (Kawai and Akira, 2007).

TLRs are expressed in various cell types in the central nervous system, including microglia (TLR1-9), astrocytes (TLR1-5; TLR9), and neurons (TLR3). Neurodevelopment and neurodegeneration are associated with TLR activation. (Yuan et al., 2010; Okun et al., 2011). TLRs are expressed in the developing as well as the adult brain and play a significant role in plasticity (Larsen et al., 2007; Kaul et al., 2012). TLR8 activation induces neuronal apoptosis through inhibition of neurite outgrowth (Ma et al., 2006), while induction of TLR3-mediated immunity during gestation inhibits cortical neurogenesis and synaptic transmission (Yuan et al., 2010). TLRs are also potential therapeutic targets against neuroinflammation. A number of these compounds are currently undergoing different phases of clinical trials (Kanzler et al., 2007).

mRNA expression of TLRs in peripheral blood mononuclear cell (PBMC) was used to detect changes in innate immunity in many physical diseases including asthma (Sykes et al., 2013), chronic hepatitis B (Huang et al., 2013) or sepsis (Cejkova et al., 2012). The relationship between TLRs and psychiatric diseases were investigated before. A systematic analysis of TLRs revealed an enhanced response in TLR2 and TLR4 activation in patients with schizophrenia or bipolar disorder (McKernan et al., 2011a). In autism, an

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enhanced TLR2 and TLR4 responsivity to its ligand can be measured, while TLR9 showed a decreased response in the blood (Enstrom et al., 2010). Alcohol abuse was also found to be associated with the TLR2, 3 and 4 complex (Kanuri et al., 2009; Crews et al., 2013). In clinical depression, translocation of the Gram-negative enterobacteria, which contain LPS in their bacterial wall, was thought to be associated with the onset of a number of inflammatory and oxidative/nitrosative stress pathways and play an important role in major depressive disorder (Maes et al., 2008). It has been demonstrated that after stress exposure or during certain episodes of depression, an innate immune response is strongly activated (Raison et al., 2006). Elevated TRIF and MYD88, intracellular adapter proteins for TLR signaling, were found in peripheral blood mononuclear cells isolated from depressed patients when compared to healthy subjects (Hajebrahimi et al., 2014). However, the systematic analysis of TLR expression in humans is still absent. Therefore, the present study aims to investigate whether TLRs expression levels are altered in patients with major depressive disorder, and how these changes correlate with the severity of depressive symptoms.

2. Method

2.1. Subjects

Thirty outpatients or inpatients with major depressive disorder and 29 healthy controls were recruited from August 2013 to December 2013 at Kaohsiung Chang Gung Memorial Hospital Medical Center in Taiwan. Institutional Review Board approval was obtained from the hospital ethics committee (101-5012A3).

Thirty patients with major depression were evaluated by one psychiatrist using the Structured Clinical Interview for DSM-IV Axis I Disorders. The severity of depression was assessed using the 17-item Hamilton Depression Rating Scale (HAMD-17) (Hamilton, 1960) by the same psychiatrist. Patients who had alcohol dependence or any immune inflammatory disease history were excluded from the study. The 29 healthy controls, who had neither a personal history nor a first degree relative with psychiatric disorder, were recruited from community. The Chinese health questionnaire-12 was done to assess the healthy control group by the same psychiatrist to rule out psychiatric disease according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria.

All patients received blood pressure checks, chest X-rays, electrocardiogram examinations, and routine blood tests in order to exclude any chronic physical illness including heart, lung, liver, kidney, or metabolic diseases. They were also checked for acute infections or allergic reactions; moreover, they reported not taking any antidepressants for at least 1 week before they entered into this study.

2.2. Real-Time Polymerase Chain Reaction (RT-PCR) analysis

Venous blood (5 mL) samples were drawn into PAXgene Blood RNA Tube between 8:00 am and 10:00 am, after the patients had fasted for 9 h. The tubes were stored in a −80 °C refrigerator immediately after collection and remained there until they were assayed. RNA was isolated using TRIzol reagent, and RT-PCR was performed using following sets of primers (López et al., 2012): TLR1 (sense 5′-GGGTCAGCTGGACTTCAGAG-3′, anti-sense 5′-AAAATCCAAATGCAGGAACG- 3′); TLR2 (sense 5′-TCAGCCTCTCCAAGGAAGAA-3′, anti-sense 5′-AATGTTCAAGACTGCC-CAGG-3′); TLR3 (sense 5′-AGCCTTCAACGACTGATGCT-3′, anti-sense 5′-TTTCCA-GAGCCGTGCTAAGT-3′); TLR4 (sense 5′-TGAGCAGTCGTGCTGGTATC-3′, anti-sense 5′-CAGGGCTTTTCTGAGTCGTC-3); TLR5 (sense 5′-GGAACCAGCTCCTAGCTCCT-3′, anti-sense 5′-AAGAGGGAAACCCCAGAGAA-3′); TLR6 (sense 5′-CCCTTTAGGATAGC-CACTGC-3′, anti-sense 5′-CTCACAATAGGATGGCAGGA-3′); TLR7 (sense 5′-CCTTGA-GGCCAACAACATCT-3′, anti-sense 5′-GTAGGGACGGCTGTGACATT-3′); TLR8 (sense 5′-TCCTTCAGTCGTCAATGCTG-3′, anti-sense 5′-CGTTTGGGGAACTTCCTGTA-3′); and TLR9 (sense 5′-GGACACTCCCAGCTCTGAAG-3′, anti-sense 5′-TTG GCTGTGGA-TGTTGTTGT-3′). The QuantiTect Reverse Transcription kit (Qiagen) was used for the reverse transcription of RNA. The PTC-200 apparatus (BioRad) and the QuantiTect SYBR Green PCR kit (Qiagen) were applied for the complete analysis. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as a housekeeping gene to assess the relative abundance of mRNA.

2.3. Statistical analysis

Outliers of TLRs were excluded initially based on a boxplot outlier detection rule. All results are presented as means ± standard deviation. Chi-square was used to compare the difference between sexes. Student's t-test was used to compare the difference of age and BMI. Analysis of covariance (ANCOVA) with age, and BMI adjustment was used to compare the difference of TLRs expression levels. Multiple linear regression (stepwise method) was used to judge factors that correlates with HAMD-17 scores. All statistical analyses were performed using Statistical Product and Service Solutions (SPSS), version 22. For each test, $p < 0.05$ was considered significant.

3. Results

3.1. Baseline characteristics

Table 1 shows baseline characteristics regarding participant sex, age, and BMI (depressive patients: 11 male/19 female, mean age 45.8 ± 10.02 years, BMI 22.96 ± 3.7 kg/m^2; health controls: 11 male/18 female, mean age 42.72 ± 12.49 years, mean BMI 22.58 ± 2.93 kg/m^2). There were no significant differences in the mean age, sex, or BMI between the two groups. Duration of illness and 17-item Hamilton Depression Rating Scale in patients with major depressive disorder are also shown Table 1.

3.2. Differential TLR mRNA expression in peripheral blood from patients with major depressive disorder and healthy controls

We analyzed TLR mRNA expression and GADPH was used as a housekeeping gene to assess the relative abundance of mRNA in peripheral blood. Similar TLR expression profiles, but differences in transcript levels, were observed in depressive patients as compared to healthy controls (Table 2). Quantitative analysis revealed significantly higher TLR3, 4, 5 and 7 mRNA expression levels in patients with major depression (TLR3: patients 1.57 ± 0.82, controls 1.08 ± 0.86, $F(1,50)=4.2$, $p=0.046$; TLR4: patients 1.75 ± 1.18, controls 1.26 ± 0.85, $F(1,53)=4.31$, $p=0.043$;TLR5: patients 2.31 ± 1.96, controls 1.14 ± 0.77, $F(1,51)=7.83$, $p=0.007$; TLR7: patients 1.54 ± 0.66, controls 1.01 ± 0.48, $F(1, 50)=10.25$, $p=0.002$), whereas no significant differences for TLR2, 8, or 9 were observed. In contrast, significant lower expressions of TLR1 and TLR6 in patients with major depressive disorder were found, compared to healthy controls (TLR1: patients 1.73 ± 1.18, controls 2.97 ± 2.37, $F(1, 50)=9.55$, $p=0.003$; TLR6: patients 1.60 ± 1.25, controls 2.86 ± 2.35, $F(1, 51)=8.55$, $p=0.005$).

Table 1
Demographic findings and clinical data of health controls and patients with major depressive disorder.

	Major depression	Healthy controls	*p* Value
Age (years)	45.8 ± 10.02	42.72 ± 12.49	0.30
male	43.18 ± 8.92	46.55 ± 14.73	0.53
female	47.32 ± 10.54	40.39 ± 10.68	0.055
Gender (M/F)	11/19	11/18	0.92
BMI	22.96 ± 3.70	22.58 ± 2.93	0.67
male	21.56 ± 2.64	24.13 ± 3.34	0.06
female	23.77 ± 4.03	21.63 ± 2.26	0.055
HAMD-17	27.83 ± 4.97	–	–
male	28.36 ± 6.41	–	–
female	27.52 ± 4.08	–	–
Duration of illness (years)	4.25 ± 3.59	–	–
male	3.91 ± 3.93	–	–
female	4.37 ± 3.54	–	–
Smoking	12	3	–
Education duration (year)	11.67 ± 3.14	15.07 ± 3.18	–
Antidepressant use History			
None	3	–	–
SSRI	11	–	–
SNRI	10	–	–
NDRI	6	–	–

Data are presented as mean ± S.D.
*p Value < 0.05; HAMD-17 = 17-item Hamilton Depression Rating Scale; BMI, body mass index.