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Possible association of Toll-like receptor 9 polymorphisms with cytokine levels and posttraumatic symptoms in individuals with various types of orthopaedic trauma: Early findings

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ABSTRACT

Background: Although TLR9 polymorphisms may be associated with cytokine dysregulation, its role in regulation of cytokines due to bodily trauma or in relation to acute stress symptoms or posttraumatic stress symptoms (ASS/PTS) has not been evaluated.
Aims: To assess serum cytokine levels and levels of ASS and PTS in relation to four common TLR9 single-nucleotide polymorphisms (SNPs) in individuals with various types of orthopaedic trauma.
Methods: Forty-eight accident-injured individuals, aged 20–60 years were studied. Serum cytokine levels and TLR9 SNPS (1486T/C, 1237T/C, 1174G/A and 2848G/A) were assessed together with intensity of ASS and PTS symptoms.
Results: Statistically significant higher serum levels of IL-12 and IL-1β ($p < .05$) were found in individuals heterozygous for TLR9-1237 (TC) than in individuals expressing the most common TLR9-1237 type (TT), while differences in levels of IL-6 were not significant. Also, marginally significant levels of IL-6 were found in individuals expressing the common TLR9-1174 (GG) compared with individuals homozygous (AA) or heterozygous (GA) for this SNP. They also had non-significant higher intensity of ASS symptoms. A trend of higher PTS levels in individuals expressing the most common type TLR9-1174 (GG) was found, contrary to homozygous (AA) and heterozygous individuals (GA).
Conclusions: The results of this pilot study suggest that accident-injured individuals with certain TLR9 polymorphisms express higher levels of pro-inflammatory cytokines (IL-1β, IL-6 and IL-12). The associations of TLR9 SNPSs with increased risk of ASS or PTS should be further studied in larger groups of such patients.

Introduction

Toll-like receptors (TLRs) are an evolutionarily conserved family of type I trans-membrane receptors, expressed on immune effector cells including T and B lymphocytes, and on macrophages [1]. It is activated by various types of bacteria, viruses and fungi, but also by self antigens [1–3]. TLR activation leads to recruitment of adaptor molecules such as MYD88, which triggers the cascade of signalling pathway and the activation of transcription factors (e.g., nuclear factor k B-cell), of mitogen activated protein kinases [1] and secretion of Th1, Th2 and regulatory cytokines [1,2].

Cytokine production and secretion upon activation may be associated with polymorphisms in TLR genes [1,4]. TLR9 expression is upregulated following body trauma and results in increase of pro-inflammatory cytokines [3,4]. Some endogenous ligands (e.g., heat shock proteins or fragments of extracellular matrix proteins), which may be released after cell damage induced by stress signals, may stimulate TLR9 and trigger inflammatory responses [4,5].

Polymorphisms in TLR genes may shift balance between pro- and anti-inflammatory cytokines, modulating the risk of chronic inflammation [5]. For example, a previous study demonstrated higher risk of sepsis and multiple organ failure in patients suffering blunt trauma who showed expression of certain TLR9 SNPs [4].

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Recently, TLR9 polymorphisms have been associated with cytokine dysregulation and autoimmune diseases [6], and with increased risk of asthma [7].

Evidence exists that acute stress symptoms (ASS), including intrusive thoughts, avoidance, physiological hyperarousal and disassociation [8], may be a precipitating factor for posttraumatic stress symptoms (PTS) or posttraumatic stress disorder (PTSD) development [9–11], especially after traumatic injuries [12], although contrasting results also were reported [13,14]. PTSD and PTS severity were previously found related to immune dysregulation [15,16]. Especially, increased levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-1β [17–22], chemokines like IL-8 and anti-inflammatory cytokines like TGF-beta [20], and in some studies, decreased levels of regulatory cytokines (i.e., IL-4, IL-10) [20,22] were found in individuals with PTSD or high PTS. Several studies found no relation or opposite associations between certain cytokines levels and ASS or PTS [20,23]. Also, it was previously suggested that altered levels of cytokines play a role in the aetiology of ASS or PTS through their effects on the central nervous system (CNS) [15,16,20].

Accumulating evidence from family, twin, and molecular studies suggest that genetic factors may contribute to the likelihood of developing PTS or PTSD upon exposure to traumatic events [24,25]. To date, most molecular genetic studies of PTSD have focused on the genes of the dopaminergic and serotonergic systems, or the hypothalamic-pituitary-adrenal axis components and the locus coeruleus/noradrenergic systems [26]; polymorphism in TLRs has not been assessed in this regard.

The present study is based on the evidence that severe body trauma is associated with inflammatory processes and that peptides released from damaged tissues may also activate TLR9, and cause dysregulation in secretion of cytokines [4,27]. Altered secretion of certain cytokines may be a precipitating factor for development of PTS [15,16], and possibly interact with high ASS [20]. No studies up to date have assessed polymorphisms in TLR genes in relation to ASS or PTS. Accordingly, we assessed serum cytokine levels and common polymorphisms in four TLR9 SNPs, two located in the promoter, namely 1486T/C and 1237T/C, one in the intron 1 1174G/A, and one in the exon 2 2848G/A, and related them to ASS and PTS symptoms.

Methods

Participants

This study is part of a larger study assessing patterns of cytokine levels in relation to ASS and PTS in posttraumatic injury [20]. Forty-eight accident-injured individuals with various types of fractures hospitalized in orthopaedic surgery departments of two major hospitals in northern Israel participated in the study. Inclusion criteria were age 20–60 and ability to communicate in Hebrew. Individuals with known psychiatric diagnosis or inflammatory, endocrine, or clinically significant chronic or acute illness were excluded.

Demographic and injury-related characteristics are presented in Table 1. Participants' mean age was 39 years, mean years of education was about 13, the majority were male, and the majority were employed. Type of accident varied: most were work accidents, traffic accidents and falls.

Procedure

Participants were approached at the 3rd–7th day (Time 1, T1) of hospitalization, 30 cm^3 heparinized venous blood samples were drawn and participants completed the questionnaires. Twenty-six participants agreed to fill out a follow-up questionnaire one month later (T2). The study was approved by the Institutional Review Board at each institute.

Table 1
Demographic characteristics of the study group ($N = 48$).

Variable		
Males (*N*, %)	34	70.8
Age, years (*M*, SD)	39.2	12.5
Range	20–60	
Education (*M*, SD)	12.7	3.1
Range	4–21	
Employed (*N*, %)	42	87.5
Religion (*N*, %)		
Jewish	28	58.3
Not Jewish	20	41.7
Financial status (*N*, %)[a]		
Good + Excellent	21	44.7
Average	18	38.3
Poor	8	17.0
Accident type (*N*, %)		
Traffic	9	18.8
Work	19	39.6
Sport	6	12.5
Falls	8	16.7
Other	6	12.4
Severity of injury[a] (*N*, %)		
Minor		14.6
Moderate	20	41.7
Serious	17	35.4
Severe	4	8.3

[a] Based on AIS scores of severity of injury (1–4).

Serum cytokine levels

A multiple analyte detection system based on fluorescent bead immunoassay was used for quantitative detection of serum cytokines IL-1β, IL-6 and IL-12. Data were collected on a FACSCalibur (Becton–Dickinson) and analyzed with FlowCytomix Pro 2.2 software – Bender MedSystems.

TLR9 polymorphism

PCR-RELP for TLR9 polymorphisms in T-1237C, and inT-1486C were performed on a Biometra TPersonal Thermal Cycler using established primers. PCR products were digested with the enzymes AflII (1486) or BstNI (1237) and were separated on 4% low melt agarose gel. M: 100 bp DNA ladder. TLR9-2848G/A and TLR9-1174G/A were tested by the RT-PCR method. SNP genotyping was performed by TaqMan allelic discrimination using the Assay-by-DesignSM SNP Genotyping Assays (Applied Biosystems).

Questionnaires

Self-report questionnaires were used to assess demographic (age, education, income, familial status) and accident-type characteristics. ASS was assessed at T1 with the Acute Stress Disorder Interview (ASDI) [9] with 19 items: dissociative symptoms (5 items), re-experiencing (6 items), avoidance (4 items), and arousal (6 items). Summed affirmative responses gave the ASS score, from 0 to 19. Cronbach's $\alpha = 0.89$.

PTS was measured by the PTSD Symptom Scale-Interview (PSS-I) [28] at T2. The 17-item PTS are rated by the participant from 0 (none at all) to 3 (5 or more times per week/very much). Summed affirmative responses gave the PTS score, from 0 to 51. Cronbach's $\alpha = 0.86$.

Severity of injury was measured by abbreviated injury scale (AIS), range from 0 (minor) to 4 (severe) [29–31]. The Abbreviated