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Review

The biological significance of TLR3 variant, L412F, in conferring susceptibility to cutaneous candidiasis, CMV and autoimmunity ☆

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ABSTRACT

Objective: Toll-like receptors, a major component of the innate immune system, play an important role in the initial response against pathogens. Genetic abnormalities in some receptors like TLR2, TLR3 and TLR4 have been associated with susceptibility to fungal and viral infections while other aberrations in TLR genes such as TLR3, TLR7 and TLR9 may predispose to autoimmunity. Recently we have shown an association of a TLR3 receptor variant, L412F, to susceptibility to chronic candidiasis, recurrent viral and bacterial infections and autoimmunity. We investigated here the biological implications of this TLR3 mutant.
Methods: To study the functional impact of the L412F variant of TLR3 we tested patients' peripheral blood mononuclear cells (PBMCs) as well as fibroblasts for secretion of cytokines in response to TLR3 ligand, candida or cytomegalovirus (CMV). In addition, the P2.1 cell line was used as a model for the TLR3 WT and L412F variant receptors function.
Results: Patient's cells carrying the L412F variant showed reduced IFNγ as well as TNFα secretion in response to stimulation with the TLR3 ligand, CMV or *Candida albicans*. Fibroblasts with the L412F variant showed decreased secretion of IFNλ in response to stimulation with both polyinosine ploycytidylic acid (Poly I:C) and CMV and P2.1 cells transfected with the L412F variant showed reduced secretion of IFN-β in comparison to cells transfected with the wild type receptor.
Conclusion: We have shown here aberrant biological responses mediated by the TLR3 variant receptor, L412F, which may explain in part susceptibility of patients to chronic candidiasis, viral infections and autoimmunity.

Contents

Abbreviations: AMD, age related macular degeneration; AIRE, autoimmune regulator gene; BCR, B cell receptor; CMCC, chronic mucocutaneous candidiasis; HSV-1, herpes simplex virus type 1; IRAK4, interleukin-1 receptor-associated kinase 4; IMEM, isocoves minimum essential medium; PAMPs, pathogen-associated molecular patterns; PHA, phytohemagglutinin; Poly I:C, polyinosine ploycytidylic acid; SEAP, serum alkaline phosphatase; SSPE, subacute sclerosing panencephalitis; TLR, toll-like receptor.

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1. Introduction

Toll-like receptors (TLRs) play an important role as sensors for viral, bacterial and fungal pathogens. These receptors are able to recognize different pathogens by conserved pathogen-associated molecular patterns (PAMPs) and thereby initiate an immune response [1,2]. TLRs also act as "bridging molecules" between innate and adaptive immunity [3]. Ligation of TLRs triggers secretion of proinflammatory cytokines such as Type I interferons, which protect against viruses but also initiate adaptive immune responses.

Genetic aberrations in TLRs and in downstream signal transduction pathway molecules were shown to be associated with susceptibility to a range of pathogens. Mice lacking TLR3 display inadequate responses to cytomegalovirus (CMV) [4]. In humans, rare null mutations in interleukin-1 receptor-associated kinase 4 (IRAK4) and MyD88, result in recessive predisposition to recurrent staphylococcal and pneumoccocal infections [5,6] while rare mutations in UNC-93B or TLR3 result in recessive predisposition to encephalitis caused by herpes simplex virus type 1 (HSV-1) [7,8]. In recent years the role for TLRs in the immune response to *Candida* and other fungal species had also been recognized. Innate immune responses to *Candida* are known to be mediated through TLR1, TLR2, TLR3, TLR4, TLR6 and downstream molecules of the TLR signal transduction pathway MyD88, as well as other receptors such as beta glucan receptor Dectin-1 [9–13]. Susceptibility to various fungal species was attributed to genetic mutations and polymorphisms in TLR receptors. Presence of TLR1 N248S and TLR6 S249P were associated with increased susceptibility to aspergillus infection after stem cell transplantation [14]. Patients with the TLR2 A753G variant showed reduced secretion of IFN-γ and IL-8, during *Candida* sepsis, and TLR4 polymorphism A299G and T399I were also found to be a risk factor for *Candida* l invasive infection [15–16]. Recently mutations in Dectin-1 [17], CARD9 [18] or LYP R620W [19] were also shown to be associated with chronic candidiasis. Chronic non-invasive candidiasis is a hallmark of chronic mucocutaneous candidiasis (CMCC) [20,21]. This is a heterogeneous group of patients who share common features including candidiasis, multiple endocrine abnormalities, as well as other autoimmune manifestations. Mutations in the autoimmune regulator gene (AIRE) was implicated in causing one type of CMCC in several well defined ethnic groups including Finns and Iranian Jews [20,21].

Aberrations in innate immunity may also be associated with autoimmunity and increased inflammation. Mutations in TLR7 and TLR9 were linked to the pathogenesis of systemic lupus erythematous in murine models [22,23], while TLR3 plays a key role in models of type 1 diabetes mellitus, and experimental autoimmune encephalomyelitis [24,25].

TLR3 recognizes viral double-strained RNA and upon ligand binding, during an infection, mediates cytokine production through the activation of the transcription factor NFkB [26]. TLR3 is localized mainly on intracellular vesicles with some cell surface expression. The structure of human TLR3 ectodomain (ECD), based on crystal structure and electron microscopy, revealed a horseshoe shape, typical of a protein containing multiple leucine-rich repeats [27]. Several genetic variants of TLR3 have been recently carefully studied. The L412F mutant of TLR3 affects a residue localized near the concave surface of the TLR3 ECD. It is predicted to alter hydrophobic interactions and affect glycosylation of neighboring residues which are critical for TLR3 function. Further, L412F affects a residue that is highly conserved from human to fish, rendering a mutation in this site very likely to be disruptive to structure and function of TLR3 [28].

Recently we have demonstrated increased prevalence of this variant receptor in a group of patients with CMCC (that had no mutations in AIRE, LYP, Dectin-1 or CARD9 genes) who suffered from chronic candidiasis, severe viral infections, especially CMV, and multiple autoimmune manifestations [29]. In this study we are showing the biological significance of this variant receptor as manifested by aberrant cytokine secretion, in both patients peripheral blood leukocytes and fibroblasts as well as in the P2.1 cell line transfected with the variant TLR3 receptor.

2. Methods

2.1. Subjects

The study was conducted with the approval of the SickKids Research Ethics Board. Genetic tests were performed with written informed consent from either a guardian of the patient or the patient. Excluded from the study were patients with a known primary immunodeficiency such as hyper IgE syndrome, SCID, acquired immunodeficiencies such as HIV infection, patients with malignancies as a primary diagnosis and patients treated with steroids or antibiotics before the appearance of chronic candidiasis.

2.2. T cell proliferation assays

To assay lymphocyte proliferation, peripheral blood mononuclear cells (PBMCs) from subjects were isolated through Ficoll-Hypaque gradient centrifugation, were incubated at 37 °C (5% CO_2) in RPMI 1640, supplemented with 10% (vol/vol) fetal calf serum (Gibco/BRL, Gaithersburg, MD USA). Cells were incubated in round-bottomed tissue culture plates for 3 days, with or without anti CD3 antibody, Poly I:C or both (InvivoGen, San Diego, CL, USA). Four hours before termination of the culture, 1 μCi of [^{3}H]thymidine was added to each well. The cells were then harvested, and samples were counted in a liquid scintillation counter.

2.3. Molecular and genetic analysis

DNA was available for genetic testing for patients that were or are currently seen by our immunology clinic. All exons and exon/introns boundaries of the TLR1, TLR2, TLR3, TLR4, TLR6, MyD88, CLEC7A (Dectin-1), AIRE, STAT3 and CD25 genes were PCR amplified and sequenced with an automated sequencer (Beckman Coulter CEQ8000) and compared with the normal genes sequences. Statistical analysis for association was determined using the Fisher exact test with appropriate degree of freedom (Prism, GraphPad software).