Concise Report

Lack of genetic association of the Toll-like receptor 4 (TLR4) Asp299Gly and Thr399Ile polymorphisms with spondylarthropathies in a Hungarian population

P. Gergely Jr1,2, A. Blazsek1, Z. Weiszhaár1, B. Pazár1 and G. Poór1,2

Objectives. Bacteria have long been suggested as aetiological factors in the genetically susceptible host in spondylarthropathies, including ankylosing spondylitis (AS) and reactive arthritis (ReA). Variability of the Toll-like receptor 4 (TLR4) gene has been shown to play a role in the inflammatory response to certain bacterial infections. We investigated whether TLR4 Asp299Gly and Thr399Ile polymorphisms contribute to the genetic background of spondylarthropathies in a cohort of Hungarian patients with AS and ReA.

Methods. DNA was obtained from patients with AS (n = 138), ReA (n = 91) and ethnically matched healthy controls (n = 140). Genotyping was carried out by polymerase chain reaction–restriction fragment length polymorphism analysis and the results were confirmed by direct sequencing.

Results. No significant differences in allele or genotype frequencies were observed between controls and either the AS patients or the ReA patients. Clinical characteristics of these groups were unrelated to the presence of any of these polymorphisms.

Conclusions. Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms do not contribute to disease susceptibility in either AS or ReA. Functional abnormalities of the TLR4 signalling pathway suggested in spondylarthropathies seem not to be genetically determined by these two common polymorphisms.

KEY WORDS: Toll-like receptor 4, Polymorphism, Ankylosing spondylitis, Reactive arthritis.

The spondylarthropathies, including ankylosing spondylitis (AS) and reactive arthritis (ReA), are an interrelated group of multisystem inflammatory disorders of unknown aetiology characterized by spine, peripheral joint or periarticular involvement and are variably associated with extra-articular manifestations. They show increased prevalence among individuals carrying the HLA-B27 gene, although the exact pathogenic significance of this association is not fully understood at present. Despite the common occurrence of ReA after a gastrointestinal or genitourinary infection and serological evidence of certain bacteria, the exact cause-and-effect relationship is still elusive. Microbial origin, although suggested by several lines of evidence, is even less supported in AS (reviewed in [1]).

The innate immune system detects the invasion of microorganisms through the Toll-like receptors (TLRs), which recognize microbial components and trigger inflammatory responses [2]. TLR4 was the first mammalian TLR identified, and mice lacking TLR4 did not respond to lipopolysaccharide, while overexpression of TLR4 was shown to cause induction of the NF-κB-dependent inflammatory pathway [3].

Growing amounts of data suggest that the ability of certain individuals to respond properly to TLR4 ligands may be impaired by single-nucleotide polymorphisms (SNPs) within TLR4 genes [4]. The Asp299Gly and Thr399Ile alleles of the TLR4 gene have been associated with increased risk of severe infections [5]. These polymorphisms have also been shown to confer risk of both Crohn’s disease [6] and ulcerative colitis [7], which are inflammatory conditions frequently associated with spondylarthropathy.

Based upon these findings, variations of the TLR4 gene may be of significance in the aetiopathogenesis of spondylarthropathies. van der Paardt et al. recently found no association between TLR4 Asp299Gly polymorphism and ankylosing spondylitis in a Dutch population [8]. In the present study we investigated the possibility whether the TLR4 Asp299Gly and Thr399Ile polymorphisms contribute to disease susceptibility in Hungarian patients with AS or ReA.

Methods

Subjects

One hundred and thirty-eight AS patients and 91 ReA patients (Table 1) were recruited from the National Institute of Rheumatology and Physiotherapy, a national referral centre in Budapest. Control subjects (n=140) included healthy blood donors from the Budapest region. All individuals were unrelated Hungarian whites. Patients with AS were diagnosed based on the modified New York Criteria [9]. The diagnosis of ReA was made by the expert opinion of a consultant rheumatologist if patients presented with the clinical picture of an asymmetrical arthritis and a preceding symptomatic urethritis or enteritis no longer than 4 weeks before the onset of arthritis. Alternative causes of

1National Institute of Rheumatology and Physiotherapy, and 2Musculoskeletal Molecular Biology Research Group, Hungarian Academy of Sciences, Budapest, Hungary.

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Correspondence to: G. Poór, 1st Department of Rheumatology and Metabolic Osteology, National Institute of Rheumatology and Physiotherapy, Frankel Leó u. 38-40, H-1023 Budapest, Hungary. E-mail: orfireum@axelero.hu

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arthropathies, such as septic arthritis, Lyme disease, crystal arthropathy, rheumatic fever, osteoarthritis, trauma, orthopaedic conditions, rheumatoid arthritis or autoimmune rheumatic diseases, had been excluded. Patients in whom joint symptoms might have been attributed to spondyloarthropathies other than ReA (e.g. psoriatic arthritis, inflammatory bowel disease-associated arthropathy, undifferentiated spondyloarthropathy) were also excluded. The study was approved by the local ethical committee (Institutional Review Board of National Institute of Rheumatology and Physiotherapy) and written informed consent was obtained from each participant.

Genotyping

Genomic DNA was obtained from 200 µl peripheral blood using the Roche High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany). Genomic DNA (1 µl) extract was used for PCR amplification in a total reaction volume of 50 µl. Genotyping for the TLR4 Asp299Gly and TLR4 Thr399Ile polymorphisms was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method with the primer pairs described by Lorenz et al. [10].

Results

There was no significant difference in allele or genotype frequencies between controls and the cohorts of AS or ReA patients for any of the Asp299Gly and Thr399Ile polymorphisms. (Table 2). We found strong cosegregation between the two SNPs (D′ = 1, r² = 0.881, P < 0.001). Hardy–Weinberg equilibrium for the two SNPs was confirmed in all cohorts. Gender, the presence of peripheral arthritis or extraskeletal manifestations and age at first complaint were unrelated to any of these two SNPs in the AS group. Similarly, in the ReA patients no significant differences in the allele frequencies for either SNP were found in the subgroups stratified for gender, disease duration, age at first complaint, type of arthritis (mono-, oligo- or polyarthritis), HLA-B27 positivity or type of the preceding infection (data not shown).

Discussion

Our results confirmed previous studies that found strong cosegregation of the Asp299Gly and Thr399Ile polymorphisms. On the other hand, we failed to find any association of any of these two common variants of TLR4 with AS or ReA in a Caucasian Hungarian population. Our results are in accordance with the recent data by van der Paardt et al., which showed no difference between genotype and allele frequencies of the Asp299Gly polymorphism in unrelated white Dutch AS patients and healthy controls [8]. Due to the relatively low sample size and low observed power values of 0.21–0.31 calculated from Table 2, we cannot conclusively exclude these polymorphisms as statistically associated alleles. However, our findings, considered together with the data of van der Paardt et al., suggest that the biological significance of these polymorphisms in the pathogenesis of spondylarthropathies is low. Further, the relatively distant location of the TLR4 gene on chromosome 9q32-q33 from the previously proposed AS susceptibility regions on chromosome 9 [12] does not support the hypothesis that TLR4 gene variants are likely to play a major role in genetic susceptibility to AS.

Growing evidence on the TLR4 receptors and their roles in infection and the common occurrence of a preceding infection in ReA raises the possibility that the pathological events in ReA may be driven in a TLR4-dependent manner. However, to our knowledge, genetic variants of TLR4 in human reactive arthritis have not been examined until the present study. Our negative findings do not exclude the importance of TLR4 in spondylarthropathies. Kyo et al. recently demonstrated that mice primed with cell wall extract from Escherichia coli (ECW) manifested acute severe arthritis after intra-articular challenge with ECW or
lipopolysaccharide, but mutant mice lacking functional TLR4 were resistant to this arthritis [13]. Furthermore, inflammation in patients with spondylarthropathy is characterized by increased TLR4 expression on peripheral blood mononuclear cells and synovial tissue [14].

Our data suggest that genetic determination by these common TLR4 variations is unlikely to play a role in the aetiopathogenesis of AS or ReA. This finding, however, does not exclude the possibility that functional abnormalities of the TLR4 or other molecules closely associated with the TLR4 signalling and the innate immune system are important in the pathogenesis of spondylarthropathies.

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