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TLR7 Gln11Leu single nucleotide polymorphism in patients with sarcoidosis

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Abstract. Sarcoidosis is a multi-factorial systemic disease with increased activity of the cellular immune components which is responsible of the formation of non-caseating granulomas in involved organs. Recent views on the etiology indicate interactions between inherited susceptibility and environmental or lifestyle factors. Concerning genes that may influence susceptibility to sarcoidosis Toll-like receptors (TLRs) may represent plausible candidates. In this present study, we investigated the X-linked TLR7 rs179008/Gln11Leu polymorphism situated on exon 3. SNP genotyping of the *TLR7* exon polymorphism was performed by TaqMan allelic discrimination using the StepOnePlus™ Real-Time PCR System (Applied Biosystems). In females, the incidence of the AT genotypes of the polymorphism was significantly lower in sarcoidosis patients compared to control subjects (P=0.0001). We could observe in control subjects a significant preponderance of the T allele of the TLR7 rs179008/Gln11Leu polymorphism compared to sarcoidosis female patients (P=0.008). In males, no significant differences between patients and controls emerged in allele frequencies of the TLR7 rs179008/Gln11Leu polymorphism. The presence of the TLR7 rs179008/Gln11Leu polymorphism in sarcoidosis may determine an alteration of TLR7 function hampering the signaling pathway involved in the onset of both cellular and humoral autoimmunity. This is consistent with the view that in some circumstances genetic mutations affecting components of the immune system can prevent systemic autoimmunity. *(Sarcoidosis Vasc Diffuse Lung Dis 2013; 30: 157-161)*

Key words: TLR7, sarcoidosis, innate immunity

Introduction

Sarcoidosis is a multi-factorial systemic disease with increased activity of the cellular immune components which is responsible of the formation of non-caseating granulomas in involved organs (1). The etiology of sarcoidosis is currently unknown. Recent views on the etiology indicate interactions between inherited susceptibility and environmental or lifestyle factors (2). It has been hypothesized that a so far unidentified, but presumably inhaled, exogenous agent induces the process in predisposed individuals (2). Concerning genes that may influence susceptibility to sarcoidosis, they may be involved in the complex cellular and molecular interactions allowing the immune system to mount tailored and effective responses. Among the potential players in the

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initiation of this process, Toll-like receptors (TLRs) may represent plausible candidates. They are a family of transmembrane proteins expressed in most immune cells and are main components of innate immunity (3). A total of ten members of the TLR family have so far been isolated in human scattered all over the genome (4). TLRs play a crucial role in the recognition of components derived from a wide range of pathogens. Furthermore, imbalanced host immune response via TLR-dependent signaling pathways may be responsible of inflammatory and autoimmune diseases (5, 6). In particular, TLR 7 has been implicated in several autoimmune disorders (7). TLR7 gene is located on chromosome X and its product was originally found to recognize imidazoquinoline compounds, such as imiquimod and resiquimod (R-848) and the guanosine analog loxoribine (8). TLR7 recognizes ssRNA derived from RNA viruses, synthetic poly(U) RNA and small interfering RNA sequences and heat shock proteins which are induced by cell damage (9). In particular, plasmacytoid dendritic cells express marked levels of TLR-7 whose stimulation leads to high level production of type I interferon (10). In the present study, we investigated the X-linked TLR7 rs179008/Gln11Leu polymorphism situated on exon 3. To date no sufficient evidence has been demonstrated for a significant association of this polymorphism with human diseases, although contrasting results have been published in the literature on systemic lupus erythematosus (11, 12). TLR7 gene is located on the X-chromosome and contains three exons. The leucine (Leu) variant encoded by the T allele of the single nucleotide polymorphism (SNP)rs179008 is located within TLR7 exon 3 and leads to the replacement of the wild allele A-encoded glutamine (Gln) at codon 11 in the protein (Gln11Leu). The variant allele is suggested to code for a functionally impaired protein (13).

Material and Methods

Study groups

One hundred-forty-nine sarcoidosis patients and 151 sex and age matched healthy controls were included in the study. Written informed consent was obtained from all subjects and the study was performed under a protocol approved by the Padua Ethical Committee. All subjects were interviewed about the course of their disease and agreed to release relevant medical records for research review. Among the 149 sarcoidosis patients, 85 were women and 64 men. All the participants in this study were Caucasian. Their ages ranged from 29 to 82 years. The number of smokers was 21% of the total. The patients included in the study were monitored regularly for at least 36 months. Diagnosis of sarcoidosis was based on clinical, radiological and histological findings, according to the American Thoracic Society (ATS), the European Respiratory Medicine Society (ERS), and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) Statement. Respiratory function tests, including single-breath diffusing capacity for carbon monoxide, were done in all patients. All patients performed chest X-ray in posterior/anterior and lateral projection and the high resolution CT scan of the chest. 36% of patients had a clinical-radiologic diagnosis, while the others 64% of patients had a histological diagnosis based on video-assisted thoracoscopy, transbronchial biopsy, cutaneous, lymph nodes or muscular biopsies. At disease onset the majority of these patients (71%) referred dyspnea, dry cough and asthenia. Extra pulmonary localizations were present at the diagnosis in 49% of patients: 25% with cutaneous involvement, 11% with lymph nodal localization, 7% with ocular involvement and 6% with liver involvement.

The radiological stages were classified according to commonly accepted criteria (Scadding score):

- Stage 0: (normal) 15% of patients;
- Stage 1: (bilateral hilar lymphadenopathy) 41% of patients;
- Stage 2: (bilateral hilar lymphadenopathy plus pulmonary infiltrates) 23% of patients;
- Stage 3: (pulmonary infiltrates) 16% of patients;
- Stage 4: (pulmonary fibrosis) 5% of patients.

TLR-7 polymorphism gene analysis

The TLR7 gene is located on chromosome Xp22.2. The TLR7 exon polymorphism we analyzed was SNP *rs179008* (*A>T*), bp 17,961 relative to start codon ATG on exon 3 (13). SNP genotyping of the *TLR7* exon polymorphism was performed by TaqMan allelic discrimination using the Assay-by-De-

sign SNP Genotyping Assays C_2259574_10 (Applied Biosystems, Foster City, CA). Both alleles were scored in one well by using Primers and TaqMan minor groove binder probes labeled with VIC and FAM dye (forward primer, 5'-CTT TCA GGT GTT TCC AAT GTG GAC-3', and reverse primer, 5'-CCC CAA GGA GTT TGG AAA TTA GGA T-3'; probes, 5'-TGA AGA GAC **A**AA TTC-3', and 5'-ACT GAA GAG AC**T** AAT TC-3'; bold characters indicate the polymorphism). PCR was conducted according to the manufacturer's protocols on StepOnePlus™ Real-Time PCR System (Applied Biosystems) using the following program: 50°C, 2 min; 95°C, 10 min; and 40 cycles at 95°C, 15 s and 60°C, 1 min.).

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA). Analyses of data were performed by applying the chi square test. A p value <0.05 was considered significant, and an odds ratio (OR) with a 95% confidence interval (CI) was calculated.

Results

The data were analyzed separately in males and females. In females, frequency of the TLR7 rs179008/Gln11Leu polymorphism differed between patients and controls. The frequencies of the variant genotypes of the polymorphism was significantly lower in sarcoidosis patients compared to control subjects, which was ascribed to an increased AT genotype frequency and decreased AA genotype frequency (OR=0.53; CI95% 0.30-0.92; p=0.0001) (Table 1). We could observe in control subjects a significant preponderance of the T allele of the TLR7 rs179008/Gln11Leu polymorphism compared to sarcoidosis female patients (T vs A: OR=0.47; CI95% 0.25-0.86; p=0.008). In males, no significant differences between patients and controls emerged in allele frequencies of the TLR7 rs179008/Gln11Leu polymorphism (P > 0.5) (Table 2).

Discussion

Sarcoidosis is a cell–mediated immunological disorder characterized by granuloma development and production of cytokines by inflammatory cells. This may implicate that the innate branch of the immune response is not implicated in this autoimmune condition. On the other hand, a large body of evidence suggests that in certain autoimmune diseases, the deregulation is not exclusively in the adaptive branch of the immune response but in the innate one as well (14). A deregulated innate response by boosting antigen presentation and suppressing regulatory

Table 1. Distribution of the different genotypes in female subjects

Genotype	Sarcoidosis patients		Healthy subjects		Total	
	n	(%)	n	Genotype	n	(%)
TT	4	(4,7)	2	(2,3)	6	(3,5)
AT	13	(15,3)	36	(41,9)	49	(28,7)
AA	68	(80,0)	48	(55,8)	116	(67,8)
Total	85		86		171	

TT vs AA: OR=1,41 CI95% 0,21-11,62 p=0,52
AT vs AA: OR=0,25; CI95% 0,11-0,56 p=0,0001

Table 2. Distribution of A and T alleles in male patients

Allele	Sarcoidosis patients		Healthy subjects		Total	
	n	(%)	n	Genotype	n	(%)
T	28	(21.9)	24	(18.5)	52	(20.2)
A	100	(78.1)	106	(81.5)	206	(79.8)
Total	128		130		258	

T vs A: OR=1.24; CI95% 0.64-2.38; p=0.49. Because the TLR-7 gene is X-linked, males are hemizygous at this locus

T cells activity can results in an overacting adaptive response against self antigens. To this regard, TLR-7 might exhibit dangerous cross-reactivity with both self-and non-self constituents resulting in direct or indirect stimulation of autoreactive T and B lymphocytes (15). In sarcoidosis, an unidentified initiating factor triggers lymphocyte activation and proliferation in the first place. The triggering antigen in sarcoidosis leads to preferential induction of autoreactive Th-1 type CD4. In particular there is clinical evidence indicating a role for some cytokines associated with a Th1 immune response, notably IFN alpha and tumor necrosis factor (TNF) alpha (16). Activation of TLR7 leads to IFN-alpha production which in turn stimulates DCs generation determining enhancement of the activity of Th1 T lymphocytes that cause cell-mediated immunity. In sarcoidosis, not only the T-cell mediated immunity, but also B-cell humoral immune responses are affected (17). Sarcoidosis frequently associates with hypergammaglobulinemia, circulating immune complexes and autoantibody production (18). To this regard, TLR7 has been involved in autoantibody production and recognition of immune-complexes containing self-DNA and/or self-RNA (19). In mouse models of systemic lupus it has been shown activation of self-reactive B-cells through a TLR7-dependent mechanism (20). It is likely that TLR7 is activated by ligands derived from damaged cells which are responsible for autoreactive B-cell proliferation, inappropriate production of autoantibodies, and the subsequent development of autoimmune disease. Furthermore, it has been documented that lupus-prone mice deficient in TLR7 showed a reduction in the severity of the disease and that blockade of TLR7 prevented autoimmune kidney and lung injury (21). Overall, the presence of the TLR7 rs179008/Gln11Leu polymorphism in sarcoidosis may determine an alteration of TLR7 function hampering the signaling pathway involved in the onset of both cellular and humoral autoimmunity. This is consistent with the view that in some circumstances genetic mutations affecting components of the immune system can prevent both organ-specific and systemic autoimmunity.

Studies of TLR7 signaling in the development of autoimmunity have revealed complex roles for this pathway (22). Therefore it is plausible that together with the TLR7 rs179008/Gln11Leu polymorphism other genetic alterations of the TLR7 signaling pathway would need to be present to determine a cumulative protective effect against sarcoidosis. This may explain the gender difference observed in our study and the fact that several patients bearing this polymorphism developed the disease. Another possible explanation for sex difference may involve the gender-specific differences of IFN-alpha levels determined by TLR7 ligands (23). It has been demonstrated that females has two times the TLR7 ligand-induced interferon-I response of males (23). This is consistent with the observed gender-related differences in immune responsiveness and sex differences in immune response genes.

Nevertheless, some limitations of the present study need to be addressed. First, we did not explore the effects of the SNP variant on the function of TLR7 protein, although previous investigations have shown a high impact of this polymorphism on protein function (13, 24). In particular, it has clearly been demonstrated that the presence of the TLR7Gln 11Leu variant was associated with decreased IFN-alpha production and impairment of the TLR7-dependent signaling pathway (24). The second limitation of our study was that X skewing might affect the interpretation of data in female. Finally, another limitation was the relative small sample size. Thus, these results should be interpreted with a certain level of caution although they were corroborated by a high statistically significant p-value.

References

1. Hamzeh N. Sarcoidosis. Med Clin North Am 2011; 95: 1223-34.
2. Chen ES, Moller DR. Sarcoidosis: scientific progress and clinical challenges. Nat Rev Rheumatol 2011; 7: 457-67.
3. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 2011; 34: 637-50.
4. Hennessy EJ, Parker AE, O'Neill LA. Targeting Toll-like receptors: emerging therapeutics? Nat Rev Drug Discov 2010; 9: 293-307
5. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010; 11: 373-84.
6. Abdelsadik A, Trad A. Toll-like receptors on the fork roads between innate and adaptive immunity. Hum Immunol 2011; 72: 1188-93.
7. Krieg AM. The toll of too much TLR7. Immunity 2007; 27: 695-7.
8. Novak N, Yu CF, Bieber T, Allam JP. Toll-like receptor 7 agonists and skin. Drug News Perspect 2008; 21: 158-65.
9. Bauer S, Pigisch S, Hangel D, Kaufmann A, Hamm S. Recognition of nucleic acid and nucleic acid analogs by Toll-like receptors 7, 8 and 9. Immunobiology 2008; 213: 315-28.
10. Kaminski MM, Ohnemus A, Cornitescu M, Staeheli P. Plasmacytoid dendritic cells and Toll-like receptor 7-dependent signalling promote

efficient protection of mice against highly virulent influenza A virus. J Gen Virol. 2012;93:555-9.
11. Sánchez E, Callejas-Rubio JL, Sabio JM, et al. Investigation of TLR5 and TLR7 as candidate genes for susceptibility to systemic lupus erythematosus. Clin Exp Rheumatol 2009; 27: 267-71.
12. Lee YH, Lee HS, Choi SJ, Ji JD, Song GG. Associations between TLR polymorphisms and systemic lupus erythematosus: a systematic review and meta-analysis. Clin Exp Rheumatol 2012; 30: 262-5.
13. Askar E, Ramadori G, Mihm S. Toll-like receptor 7 rs179008/Gln11Leu gene variants in chronic hepatitis C virus infection. J Med Virol 2010; 82: 1859-68.
14. Modlin RL. Innate immunity: ignored for decades, but not forgotten. J Invest Dermatol 2012; 132: 882-6.
15. Netea MG, Wijmenga C, O'Neill LA. Genetic variation in Toll-like receptors and disease susceptibility. Nat Immunol 2012; 13: 535-42.
16. Facco M, Cabrelle A, Teramo A, et al. Sarcoidosis is a Th1/Th17 multisystem disorder. Thorax 2011; 66: 144-50.
17. Lee NS, Barber L, Akula SM, Sigounas G, Kataria YP, Arce S. Disturbed homeostasis and multiple signaling defects in the peripheral blood B-cell compartment of patients with severe chronic sarcoidosis. Clin Vaccine Immunol 2011; 18: 1306-16.
18. Weinberg I., Vasiliev L., Gotsman I. Anti-dsDNA antibodies in sarcoidosis. Semin Arthritis Rheum 2000; 29: 328-31.
19. Green NM, Marshak-Rothstein A. Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. Semin Immunol 2011; 23: 106-12.
20. Lau CM, Broughton C, Tabor AS, et al. RNA-associated autoantigens activate B cells by combined B cell antigen receptor/Toll-like receptor 7 engagement. J Exp Med 2005; 202: 1171-7.
21. Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RA, Shlomchik MJ. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity; 25: 417-28.
22. Chiang CY, Engel A, Opaluch AM, et al. Cofactors required for TLR7- and TLR9-dependent innate immune responses. Cell Host Microbe 2012; 11: 306-18.
23. Khan N, Summers CW, Helbert MR, Arkwright PD. Effects of age, gender, and immunosuppressive agents on in vivo toll-like receptor pathway responses. Hum Immunol 2010; 71: 372-6.
24. Oh DY, Baumann K, Hamouda O, et al. A frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression. AIDS 2009; 23: 297-307.