HLA-B*39 Allele Confers Susceptibility to Osteoarticular Complications in Human Brucellosis

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ABSTRACT. ***Objective.*** To determine the contribution of HLA gene polymorphism toward susceptibility to osteoarticular focal forms of human brucellosis.

Methods. A total of 57 patients with brucellosis, of whom 23 had osteoarticular complications, and 73 healthy volunteers were genotyped for HLA class I and class II antigens by a polymerase chain reaction-sequence specific primer technique.

Results. The HLA-B*39 allele was present in significantly more patients with osteoarticular complications than in the other patients (35% vs 3%; p = 0.0006, OR 15.684, 95% CI 3.453–71.231), or in the controls.

Conclusion. The increased presence of the HLA-B39 genotype in patients with brucellosis with clinical osteoarticular manifestations suggests that this genotype confers susceptibility to developing severe osteoarticular focal forms of the disease. (J Rheumatol 2003;30:1051–3)

Key Indexing Terms:
HUMAN BRUCELLOSIS HLA-B OSTEOARTICULAR COMPLICATIONS

Brucellosis is a zoonosis transmitted to humans caused by a gram negative organism of the *Brucella* genus. It is an endemic infectious disease in Spain, with 587 cases in Andalucia in 1997, or 8.02 cases per 100,000 inhabitants[1].

When brucellosis affects a specific organ or tissue it is called focal brucellosis or complicated brucellosis, and it occurs in 1% to 30% of patients. The most common complication is osteoarticular involvement, affecting 20–85% of cases. Over 80% of these osteoarticular complications involve spondylitis, especially lumbar, or sacroiliitis. Other complications such as monarthritis, oligoarthritis, and bursitis also occur to a lesser degree.

Although the best known diseases associated with the HLA genes are the rheumatic diseases of unknown origin, such as rheumatoid arthritis and ankylosing spondylitis, a group with articular involvement is known to be associated with bacterial infections and the HLA genes. Examples of such infections include S*higella*, *Salmonella*, Y*ersinia*, or *Chlamydia*, which can result in Reiter's syndrome[2].

With regard to the infectious organism *Brucella*, only a few studies have addressed the possible association between genes of the major histocompatibility complex (MHC) and susceptibility to brucellosis or its complications, and these were undertaken with nonmolecular techniques[3-5]. We recently proposed that persons infected with *Brucella* who also have the tumor necrosis factor-α-308.2 allele are susceptible to developing the disease but not its complicated forms, this susceptibility being independent of the HLA-DR3 allele[6].

We investigated the association between the HLA class I and class II genes and the osteoarticular focal forms of brucellosis, the most common complication of this disease.

MATERIALS AND METHODS

Patients and controls. A total of 57 Spanish patients with brucellosis diagnosed by the Infectious Diseases Unit of Carlos Haya Regional Hospital in Malaga, Spain, were included in the study. Of these, 23 (40%) had osteoarticular complications (13 men, 10 women). A control group was composed of 73 healthy volunteers matched for age and sex from the same geographical area as the patients.

The diagnosis of brucellosis was established according to one of the following criteria: first, isolation of *Brucella* spp. in blood or any other body fluid or tissue sample or, second, the presence of a compatible clinical picture together with the observation of specific antibodies at significant titers or seroconversion. Significant titers were considered to be Wright seroagglutination ≥ 1/160 or Coombs' antibrucella test ≥ 1/320. Osteoarticular complications were considered to be those showing obvious inflammatory signs (heat, redness, swelling, pain, or functional disability) in any peripheral joint, or unrelieved pain at rest in any deep joint with radiographic and/or gammagraphic evidence of abnormality.

For 38 patients (66%), the diagnosis of brucellosis was established by isolating *Brucella melitensis* in blood cultures, tissue, or synovial fluid. For the remaining 19 patients (33%) the diagnosis was made by clinical-serological means. Thirty-four patients (60%) had acute uncomplicated disease and the other 23 (40%) presented some type of osteoarticular complication. These included 9 (39%) spondylitis, 4 (17%) sacroiliitis, 3 (13%) oligoarthritis, 5 (22%) monarthritis, one (4%) osteitis, and one (4%) osteomyelitis. All patients and controls gave informed consent.

HLA typing. DNA was isolated from anticoagulated peripheral blood mononuclear cells using standard techniques. HLA class I and class II typing was carried out for all subjects by polymerase chain reaction ampli-

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fication techniques with sequence-specific primers (PCR-SSP) (One Lambda, Inc., Canoga Park, CA, USA.)

Statistical analysis. The data were analyzed using the chi-square test with Yates' correction or Fisher's exact test. P values lower than 0.05 were considered significant. The magnitude of associations was estimated by odds ratios with 95% confidence intervals (OR, 95% CI) by the Woolf-Haldane method.

RESULTS

There were no significant differences between the patients, either with or without complications, and the controls in the distribution of the HLA-A alleles (data not shown). The most noticeable differences, although not significant, were the increased frequencies of the HLA-A*11 allele in patients without focal forms compared to the controls, 26% versus 5%, respectively, and the HLA-A*68 allele in patients without focal forms compared to the controls, 20% versus 3%, respectively.

The most important findings concerned the frequencies of the locus B alleles (Table 1). Surprisingly, despite the known association between HLA-B*27 and osteoarticular complications in other forms of arthritis, there was no increase in the frequency of the HLA-B*27 allele in the patients with osteoarticular complications compared to the healthy controls, 4% versus 8%, respectively. There was, however, a notable increase in the frequency of the HLA-B*39 allele in the patients with osteoarticular involvement compared to controls, 35% versus 3%. This difference was statistically significant (p = 0.0006, OR 15.684, 95% CI 3.453–71.231). There was no difference between patients without complicated forms and the controls for this B locus.

The distribution of frequencies for the alleles of the HLA-C, HLA-DR, and HLA-DQ loci showed no significant intergroup differences (data not shown).

Table 1. Frequency of the HLA-B class I antigens in patients with brucellosis with and without osteoarticular complications and in healthy controls.

B	Osteoarticular Complications, n = 23 (%)	No Complications, n = 34 (%)	Controls, n = 73 (%)	p
7	5 (22)	7 (20)	10 (14)	NS
8	4 (17)	3 (9)	7 (9)	NS
64	2 (9)	2 (6)	2 (3)	NS
65	2 (9)	5 (15)	2 (3)	NS
62	3 (13)	3 (9)	6 (8)	NS
27	1 (4)	1 (3)	6 (8)	NS
38	1 (4)	0 (0)	9 (12)	NS
39	8 (35)	1 (3)	2 (3)	0.0006
60	2 (9)	5 (15)	2 (3)	NS
44	8 (35)	12 (35)	19 (26)	NS
49	1 (4)	0 (0)	8 (11)	NS
50	2 (9)	2 (6)	5 (7)	NS
51	2 (9)	3 (9)	7 (9)	NS
58	1 (4)	1 (3)	1 (1)	NS

DISCUSSION

Different studies of brucellar arthritis have produced heterogeneous results. Although one study in patients with *B. abortus* and articular manifestations showed an association with HLA-B27[3], this has not been confirmed in later studies. Our study in patients from the south of Spain with *B. melitensis* was undertaken with molecular biology techniques to type the HLA class I and class II genes, and showed a high presence of the HLA-B*39 allele (split of HLA-Bw16) in those patients with brucellar arthritis. These aspects of ethnic group, *Brucella* strain, geographic region, and technique employed for HLA typing could explain the differences in our results compared to previous studies[3,4].

The association of the HLA-Bw16 antigen was originally reported in Caucasian patients with ankylosing spondylitis who were HLA-B27 negative. The presence of the HLA-B39 allele is increased in Caucasian patients with pauciarticular juvenile arthritis and those with psoriatic arthritis[7-9]. Not only do these studies support the theory of an association between HLA-B39 and the seronegative spondyloarthropathies, but another recent study indicates HLA-B39 as a prognostic risk factor for disease progression of early stage psoriatic arthritis[10].

Among the currently proposed hypotheses regarding the association between HLA-B27 and the spondyloarthropathies, the "arthritogenic" peptide model postulates that a bacterial infection produces a peptide that is presented in the context of the HLA-B27 molecule to cytotoxic T lymphocytes. This results in a primary response of the cytotoxic T lymphocytes, which respond via a cross-reaction with a structurally similar peptide, derived from articular tissue, which is also presented by the HLA-B27 molecule[11].

Given the similarities between HLA-B27 and HLA-B39, this model could well be extrapolated to the association between HLA-B39 and brucellar arthropathy. The major subtype of HLA-B39 shares Glu at position 45 and Cys at position 67 with HLA-B27. These polymorphic amino acid residues constitute the peptide anchoring point to the B pocket in the peptide binding groove, in which the only Arg residue at position 2 (P2) is located in the binding-motif of the HLA-B27[12]. As with HLA-B27, the positively charged amino acids including Arg are the dominant residues at P2 for the B38 and B39 alleles. This would all seem to suggest that both HLA-B27 and HLA-B39 recognize a group of overlapping peptides[13]. As a consequence of the similarity between both molecules, we propose that the increased frequency of the HLA-B*39 allele could have the following physiopathological mechanism: the HLA-B39 molecule presents the hypothetical arthritogenic peptides generated after infection with *B. melitensis*. These peptides could have the same, or at least a very similar, structural sequence as certain peptides derived from articular tissue, so that on being presented in the context of the B39 molecule, the cytotoxic T lymphocytes generate an inflammatory immune

response leading to immunologically mediated osteoarticular complications, and can therefore be included within the category of "reactive arthritis." However, given our sample size, with just 9% reactive arthritis, it is not presently possible to determine whether this allele is or is not associated with this type of arthritis. The association, therefore, has been established for all the osteoarticular forms of human brucellosis.

Thus, our results show for the first time an association between HLA antigens and osteoarticular complications after infection with *B. melitensis*. Determining genetic markers in this disease might enable us to know which patients may be susceptible to developing more severe forms of the disease. In summary, pending larger studies, we suggest that the HLA-B*39 allele is a marker of disease susceptibility in the complicated focal osteoarticular forms of human brucellosis.

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