Influence of HLA-B*5703 and HLA-B*1403 on Susceptibility to Spondyloarthropathies in the Zambian Population

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ABSTRACT. ***Objective***. To analyze the distribution of HLA-B alleles and to investigate their contribution in the susceptibility to spondyloarthropathies (SpA) in a sample population from Zambia, in order to determine a relationship between some HLA-B alleles and development of ankylosing spondylitis (AS), reactive arthritis (ReA), or undifferentiated SpA (uSpA).

Methods. We selected 72 patients with SpA and found that 46 had uSpA, 23 ReA, and 3 AS. We also selected 92 matched controls; 55 of these had human immunodeficiency virus type I (HIV-I) infection.

Results. We found a significant increase in the rate of uSpA and ReA with features of Reiter's syndrome (RS) in HIV-positive individuals who carried the HLA-B*5703 allele ($p_c < 0.0001$ and $p_c < 0.001$, respectively). Among the significant new findings identified were the presence of B*1403 in 2 of the 3 AS patients ($p_c < 0.05$, OR 47), confirming previous data in the Togolese population.

Conclusion. The presence of B*5703 and HIV infection may not affect susceptibility to AS and ReA, but they do show an important influence in uSpA and RS. Our findings confirm that HLA-B*1403 is the only factor to increase the risk of AS in a sub-Saharan African population, whereas HLA-B27 was virtually absent in patients with AS. (First Release Oct 15 2008; J Rheumatol 2008; 35:2236–40; doi:10.3899/jrheum.080395)

Key Indexing Terms:
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The spondyloarthropathies (SpA) are a group of diseases that share certain clinical features and an association with the HLA-B27 allele. These disorders include ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis and spondylitis, enteropathic arthritis and spondylitis, juvenile-onset spondyloarthritis, and undifferentiated SpA (uSpA)[1]. The high frequency of B27 in patients with SpA, especially AS, where approximately 95% of patients with AS possess B27[2], has emerged as probably the best example of a disease association with an HLA marker, suggesting that these disorders share pathogenic mechanisms.

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The incidence of SpA in sub-Saharan Black Africans is considerably lower, probably in part because of the rarity of HLA-B27 antigens in this region[3,4]. The prevalence of B27 is < 1% among those who live in central and southern Africa, and this low frequency has been generally held to be the explanation for the scarcity of SpA among sub-Saharan Africans, although the influence of HLA-B alleles other than B27 has not been investigated thoroughly. In recent years, the incidence of SpA in sub-Saharan countries has experienced an expansion, which could be linked to human immunodeficiency virus (HIV) infection[5], but not with HLA-B27. Studies have reported the relationship between SpA and HIV infection in the Zambian and Zimbabwean populations[6-8]. We have reported that HLA-B*5703 has a protective effect against the progression of HIV infection, but patients who carry this allele are at increased risk for the development of SpA[7].

It has been suggested that the presence of other HLA-B alleles different from B27 may also increase susceptibility to AS. Studies have reported that HLA-B60 and HLA-B39 increase susceptibility to AS independently of B27 in Taiwan Chinese and Japanese patients, respectively[9,10]. We previously conducted a study in the Togolese population that showed genetic evidence for implication of HLA-B*1403 in

AS[11]. Because HLA-B*1403 was detected only in several unrelated individuals during routine typing[12], further studies are needed to clarify the role of this allele in susceptibility to AS. Confirmation of our findings in the Togolese population may support the role of the HLA-B*1403 allele in development of AS in sub-Saharan populations. We analyzed the possible contribution of HLA-B alleles in the susceptibility to different SpA in a Zambian population, and the relationship of HLA-B*5703 and HIV infection.

MATERIALS AND METHODS

Cases and controls. In total, 72 consecutive cases of SpA attending rheumatology clinics at the University Teaching Hospital and at the Maina Soko Military Hospital, Lusaka, were recruited over a 10-year period. Patients had no familial relationships. All were examined by a rheumatologist and radiographs of the pelvis and lumbar spine were obtained from all patients. SpA was diagnosed according to the criteria of the European Spondylarthropathy Study Group (ESSG)[1]. Changes in the sacroiliac joint were determined according to the New York criteria[13]. Among these 72 patients with SpA, 46 had uSpA, 23 ReA [with features of Reiter's syndrome (RS) in 7; a triad of arthritis, urethritis, and conjunctivitis], and 3 had AS. We also selected 92 controls, 37 of whom were unrelated healthy blood donors and 55 were unrelated patients infected with HIV-1 without SpA. Characteristics of patients and controls are shown in Table 1. Based on these data, we included those infected with HIV-1 as a control group. All were matched for sex, age, and ethnic origin, and as no differences existed in their HLA-B profile we were able to analyze the experimental and control populations as one. HLA-B allele distribution between HIV-positive and HIV-negative controls was similar.

Patients and controls were recruited from the same geographic area, and all subjects were of Bantu origin and thus representative of the Zambian population. The study was approved by the University of Zambia Research and Ethics Committee in 2004. All patients and controls gave oral informed consent.

HLA-B typing. A 2-ml sample of peripheral blood per subject was taken from the SpA patient group and the control groups in the University Teaching Hospital and the Maina Soko Military Hospital in Lusaka, and samples of genomic DNA were obtained. All samples were typed for HLA-B by polymerase chain reaction and sequence-specific oligonucleotide (SSO) analysis using Reli SSO typing kits (Dynal, Oslo, Norway) at the Histocompatibility Unit of Hospital Universitario Central de Asturias, Oviedo, Spain.

Statistical analysis. For the case-control study, differences in HLA-B phenotypic frequencies between patients and controls were estimated by direct counting and assessed using the chi-square test with Yates' correction or Fisher's exact test. The odds ratio (OR) was calculated and exact 95% confidence intervals (95% CI) were obtained. P values were corrected (p_c) by multiplying by the number of comparisons (23 HLA-B alleles).

RESULTS

The HLA-B allele frequencies in each patient and control are shown in Table 2. We reexamined a previous study, where we report that HLA-B*5703 seems to be a protective allele against the progression of HIV infection and could influence the increased incidence of SpA observed in the Zambian population[7]. In accord with our previous results, we observed that HLA-B*5703 was increased in ReA and uSpA patients (17.4% vs 5.9%, $p < 0.05$; 25% vs 5.9%, $p_c < 0.0005$, OR 5.24, 95% CI 2.29–12.19, respectively). Moreover, one significant new finding was the presence of B*1403 in 2 of the 3 AS patients (66.6%; $p_c < 0.05$, OR 47, 95% CI 17.8–123.9), and its absence in the 92 controls. B*1402, which has been predominantly described in Caucasians, was found to be absent in patients with AS. No AS patient with the B*1403 allele showed HIV infection.

We next compared the influence of HIV infection in the development of SpA together with the presence or absence of HLA-B alleles (Table 3). For this purpose, we decided to divide ReA and RS patients into 2 groups, in order to establish differences between them. First, we found an increase of uSpA and RS patients with HIV infection with respect to controls (100% vs 59.8%, $p_c < 0.0001$; and 85.7% vs 59.8% in uSpA and RS patients, respectively), showing the same trend as that of HLA-B*5703 distribution. For this reason, we examined the association of SpA patients by comparing the presence of B*5703 and HIV infection. The frequency of HIV-positive subjects who carried the HLA-B*5703 allele was significantly increased in uSpA and RS patients (50% vs 4.3%, $p_c < 0.0001$, OR 22; and 57% vs 4.3%, $p_c < 0.001$, OR 29.3, in uSpA and RS patients, respectively). We

Table 1. Characteristics of patients and control groups in the Zambian study population.

Characteristics	Controls, n = 92	uSpA, n = 46	ReA, n = 23	AS, n = 3
Male, n (%)	82 (89.1)	35 (76.1)	7 (30.4)	2 (66.6)
Female, n (%)	10 (10.9)	11 (23.9)	16 (69.6)	1 (33.3)
HIV-positive, n (%)	55 (59.8)	46 (100)	10 (43.5)	—
	HIV-pos/SpA-neg, n = 55	HIV-pos/SpA-pos, n = 56	HIV-neg/SpA-pos, n = 16	Healthy controls, n = 37
Mean age at onset of SpA, yrs	NA	26.67 ± 6.48	34.1 ± 12.48	NA
Mean age at onset of HIV, yrs	31.3 ± 26.26	28.96 ± 5.53	NA	NA
Duration of ApA at presentation, yrs	NA	3.76 ± 2.96	1.7 ± 0.9	NA
Time since HIV diagnosis, yrs	4.39 ± 3.12	4.27 ± 2.90	NA	NA

uSpA: undifferentiated spondyloarthropathies; AS: ankylosing spondylitis; ReA: reactive arthritis; HIV: human immunodeficiency virus; NA: not applicable. Values are mean ± SD.

Table 2. Distribution of HLA-B alleles in patients with different SpA and control group.

Allele	No. (%) AS Patients (2n = 6)	No. (%) ReA Patients (2n = 46)	No. (%) uSpA Patients (2n = 92)	No. (%) Controls (2n = 184)
B*7	—	2 (4.3)	5 (5.4)	16 (8.7)
B*8	1 (16.6)	1 (2.2)	1 (1.1)	4 (2.2)
B*13	—	—	1 (1.1)	3 (1.6)
B*1401	—	1 (2.2)	—	3 (1.6)
B*1402	—	1 (2.2)	—	4 (2.2)
B*1403	2 (33.3)*	1 (2.2)	—	—
B*1503	—	—	3 (3.3)	15 (8.1)
B*1510	—	4 (8.7)	7 (7.6)	14 (7.6)
B*18	1 (16.6)	1 (2.2)	5 (5.4)	10 (5.4)
B*2705	—	3 (6.5)	2 (2.2)	—
B*35	—	4 (8.7)	2 (2.2)	9 (4.9)
B*39	—	—	1 (1.1)	4 (2.2)
B*41	—	—	1 (1.1)	3 (1.6)
B*42	—	5 (10.9)	8 (8.7)	17 (9.2)
B*44	—	2 (4.3)	1 (1.1)	11 (5.9)
B*45	—	6 (13)	5 (5.4)	12 (6.5)
B*49	—	1 (2.2)	2 (2.2)	1 (0.5)
B*51	1 (16.6)	1 (2.2)	3 (3.3)	4 (2.2)
B*5301	1 (16.6)	—	7 (7.6)	14 (8.1)
B*5702	—	1 (2.2)	2 (2.2)	2 (1.1)
B*5703	—	8 (17.4)**	23 (25)**	11 (5.9)
B*58	—	3 (6.5)	5 (5.4)	21 (11.4)
B*81	—	1 (2.2)	8 (8.7)	6 (3.3)

* AS versus controls, $p_c < 0.05$, OR 47, 95% CI 17.8–123.9. ** ReA versus controls (17.4% vs 5.9%), uncorrected $p < 0.05$; for uSpA versus controls (25% vs 5.9%), $p_c < 0.0005$, OR 5.24, 95% CI 2.29–12.19.

Table 3. HIV influence in SpA development with respect to presence of HLA-B alleles.

Status	Allele	Controls, n = 92 (%)	uSpA, n = 46 (%)	ReA, n = 16 (%)	RS, n = 7 (%)	AS, n = 3 (%)
HIV-positive	B*5703	4 (4.3)	23 (50)*	3 (18.7)	4 (57.1)*	—
	B*1403	—	—	—	1 (14.3)	—
	B*2705	—	2 (4.3)	1 (6.25)	2 (28.6)**	—
HIV-negative	B*5703	7 (7.6)	—	1 (6.25)	—	—
	B*1403	—	—	—	—	2 (66.6)***
	B*2705	—	—	—	—	—

RS: Reiter's syndrome. * uSpA versus controls (50% vs 4.3%), $p_c < 0.0001$, OR 22, 95% CI 6.9–69.9; for RS vs controls (57.1% vs 4.3%), $p_c < 0.001$, OR 29.3, 95% CI 4.8–177.7. ** RS versus controls (28.6% vs 0%), $p_c < 0.005$, OR 1.4, 95% CI 0.87–2.2. *** AS versus controls (66.6% vs 0%), $p_c = 0.001$, OR 3, 95% CI 0.61–14.86.

also found the presence of B2705 in SpA patients (2 in RS, 1 in ReA, and 2 in uSpA), all of whom were HIV-positive, and found that the frequency of B2705 was increased only in RS patients compared with controls (uncorrected $p < 0.005$). B*27 alleles were not identified in either AS patients or controls. We found that the frequency of HIV-negative subjects who carried the HLA-B*1403 allele was significantly increased in AS patients compared to controls (66.6% vs 0%; $p_c < 0.001$). HLA-B*1403 was present in one RS patient who was HIV-positive. It is known that RS can also affect the joints in the back and cause spondylitis or sacroiliitis[4]. It has also been described that patients with chronic RS could develop AS as a delayed progression of the disease[4].

Our findings confirm that HLA-B*1403 is the only factor that has been demonstrated to increase the risk of AS in the sub-Saharan population, whereas HLA-B27 is virtually absent in AS patients. These findings should be confirmed by analyzing a larger number of patients and employing a longer followup time. Thus, the presence of B*5703 and HIV infection may not affect susceptibility to AS (and ReA), but may show an important influence in uSpA and RS.

DISCUSSION

The relationship between HLA-B27 and SpA, and AS in particular, has long been known[14,15], but the pathogenic role of this gene and its product has not been resolved. It is thought that other class I and II genes may increase susceptibility to the development of AS[9-11].

We analyzed the distribution of HLA-B alleles in different SpA patients and controls in the Zambian population. HLA-B27 is the main genetic factor in the development of SpA, but it is virtually absent in most sub-Saharan African populations[8,11], suggesting that the risk conferred by B*27 in Africans is lower than that observed in Caucasians. The B*27 allele was identified in SpA patients, 2 in RS, 1 in ReA and 2 in uSpA, all of whom were HIV-positive, but was not identified in AS patients or controls. We found an increase of B*2705 only in patients with RS. Therefore, HLA-B27 was found not to be associated with AS in the Zambian population.

The relationship between HIV infection and SpA remains controversial and few studies have experimentally addressed the role of HLA antigens in the predisposition to HIV-related SpA. We found a significant increase in the rate of HIV-positive patients with uSpA and RS who were carriers of HLA-B*5703 allele. Thus, patients with HIV infection who carry this allele are at increased risk for the development of uSpA and RS, but not of AS and ReA, where the presence of B57 and HIV infection does not show an important influence.

Additionally, we observed an interesting association between the HLA-B*1403 allotype and AS susceptibility in the Zambian population. The frequency of this allotype was increased in AS patients. The HLA-B*1403 allotype has only been reported in the African populations of Togo and Cameroon[11,12]. The prevalence of the allotype in the normal Togolese population was < 1% and it was also detected in several unrelated individuals in Cameroon. We therefore found an HLA-B*1403 distribution similar to that described in our previous studies in the Togolese population[11] that supports its role in development of AS.

It has been argued that a T-cellular response originated by the specific binding of HLA-B27 molecules and arthritogenic peptides of endogenous origin and HLA-B27 might be related to the development of SpA. If T cell recognition of specific peptides were important in susceptibility to AS, one would look for common peptide features between the association of B27 and B*1403 with disease. Accordingly, the existence of shared ligands between B*2705 and B*1403 has been described, confirming that these alleles can present common peptides[16]. However, all peptides shared by these allotypes were also found to be shared by B*1402, although those results do not exclude the existence of a mechanism of AS mediated by the specific ligands of B*2705 and B*1403[16]. To clarify the different association of HLA-B14 alleles with AS, it is necessary to investigate other aspects between subtypes, such as their interaction with the peptide-loading complex (PLC), their tendency to misfolding, or their capacity to form homodimers. For example, it is known that B*14 alleles carry a B-pocket consensus motif of B27 that includes Cys67. Thus, these alleles could originate $ß_2$-microglobulin/free heavy chain homodimers dependent on the presence of Cys67, which could be recognized for some leukocyte receptors, such as LILRA1, LILRB2, KIR3DL1, and KIR3DL2.

Our findings suggest that HLA-B*1403 may be involved in the development of AS in the Zambian population, and this confirms previous data obtained in the Togolese population. These findings should be confirmed by analyzing a larger number in a sub-Saharan population. Further, we show that the influence of HLA-B57 or the presence of HIV infection does not affect susceptibility to AS. We also make a distinction between uSpA and RS and other spondyloarthropathies, because the results suggest that the HLA-B*5703 allele confers susceptibility to the development of these diseases in Zambian patients who were HIV-positive.

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