Susceptibility to toxoplasmic retinochoroiditis is associated with HLA alleles reported to be implicated with rapid progression to AIDS

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Abstract. *Background/aims:* The frequency of HLA markers associated with rapid progression to AIDS was evaluated in Brazilian patients with AIDS exhibiting or not toxoplasmic retinochoroiditis (TRC).

Methods: 98 AIDS patients (25 with TRC, 43 with anti-*T. gondii* antibodies but without TCR, and 30 without anti-*T. gondii* antibodies and without TCR) were studied.

Results: The HLA-B35 was significantly increased in TRC group ($p = 0.0038$).

Conclusion: The presence of HLA-B35 may simultaneously predispose to progression to AIDS and TRC.

Keywords: HLA, HIV, AIDS, toxoplasmic retinochoroiditis, toxoplasma gondii

1. Introduction

Genetic polymorphisms have been described to be associated with progression to AIDS or with the development of specific disease manifestations, particularly the genes of the human leukocyte antigen (HLA) system [1–3].

The progression from HIV infection to AIDS has been strongly associated with HLA-A1-Cw7-B8-DR3-DQ2 and HLA-A11-Cw4-B35-DR1-DQ1 haplotypes, conferring high risks to rapid progression [4–7]. It has

been assumed that associations between progression to AIDS and particular HLA alleles reflect differential antigen presentation by class I or II molecules exhibiting particular motifs in the peptide binding groove [8].

Toxoplasma gondii infection is widespread in humans, being infection rates estimated from 50 to 80% in the general population of South America [9]. In some areas of Southern Brazil, the prevalence of antibodies against *T. gondii* may be as high as 71% [10,11].

Toxoplasmosis in the immunocompromised host has been mainly considered to be a reactivation of a previous latent infection, and can be life-threatening [12]. Encephalitis is the most important manifestation of toxoplasmosis in immunosuppressed patients as it causes severe damage to brain and death [13]. On the other hand, the toxoplasmic retinochoroiditis has been reported to be an important opportunistic infection of the

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eye in HIV/AIDS patients, occurring in 1% to 21% of patients with acquired systemic infection [14].

We have previously reported that susceptibility to cytomegalovirus retinitis was associated with HLA alleles related with rapid progression to AIDS [15], and the availability of genetic markers for other AIDS severe complications may discriminate patients with worsen prognosis. To further explore whether HLA markers, which have been described in association with rapid progression to AIDS may be also associated with the development of toxoplasmic retinochoroiditis, we evaluated these markers in Brazilian AIDS patients presenting or not toxoplasmic retinochoroiditis.

2. Patients and methods

2.1. Patients

The study was conducted on 98 adult HIV-infected patients (81 males) aged 21 to 59 years (median = 33) with AIDS. Twenty-five patients had toxoplasmic retinochoroiditis confirmed by retinal clinical examination by a trained ophthalmologist, based on indirect binocular ophthalmoscopy and by presence of antibody against *T. gondii* (Group 1). Two additional AIDS patient groups without toxoplasmic retinochoroidites were studied; i.e., a group of 43 patients exhibiting positive anti-*T. gondii* antibodies (Group 2), and another group of 30 patients exhibiting no anti-*T. gondii* antibodies (Group 3). The median time from AIDS diagnosis until the detection of retinochoroiditis for group 1 was four years, and the median time of follow-up for patients of groups 2 and 3 were three and four years, respectively.

2.2. Ethical aspects

The local Ethics Committee of the University Hospital of Faculty of Medicine of Ribeirão Preto and the National Brazilian Ethics Committee approved the study protocol, and informed consent was obtained from all individuals (HCFMRP-USP # 8992/2001 and CONEP # 203/2002).

2.3. Anti-*T. gondii* antibodies

The search for anti-*T. gondii* antibodies in serum was performed by indirect immunofluorescence by the method of Camargo [16] using an anti-human IgG fluorescent conjugate (Bio-Mérieux). Serum samples with > 1/16 titers were considered to be positive.

2.4. HLA typing

HLA class I antigens expressed on the surface of peripheral blood lymphomononuclear cells were typed using a microlymphocytotoxicity assay [17]. DNA, obtained from peripheral blood mononuclear cells using a salting out procedure, was used for HLA class II allele typing using PCR-amplified DNA hybridised with sequence specific oligonucleotide probes or sequence-specific primer analysis using commercial kits (One Lambda, Canoga Park, CA, and Ruprecht-Karls-Universität, Heidelberg, Germany), as previously described [18].

2.5. HLA specificities associated with the rate of progression to AIDS

Since HLA-A1, A11, B8, B35, DR3, DR1, DQ2, DQ1 antigens have been described in the literature in association with rapid progression to AIDS [4–7] in many ethnic groups, these markers were considered for analysis in the present study.

2.6. Statistical analysis

HLA antigen and HLA allele group frequencies were calculated by direct counting. The strength of the association between toxoplasmic retinochoroiditis and HLA specificities was evaluated by calculating the relative risk using the Fisher's exact test, considered to be significant at $p < 0.05$. The etiologic fraction (EF) that estimates the strength of the associations at the population level was also calculated [19].

3. Results

3.1. HLA profile according to the presence of toxoplasmic retinochoroiditis

The presence of at least three alleles associated with rapid progression to AIDS (from the literature described HLA-A11-Cw4-B35-DR1-DQ1 haplotype) was significantly increased among toxoplasmic retinochoroiditis AIDS patients in relation to patients with AIDS exhibiting anti-*T. gondii* antibodies but without toxoplasmic retinochoroiditis ($P = 0.01$, $RR = 3.04$, $EF = 0.10$). The frequency of the whole HLA-A11-Cw4-B35-DR1-DQ1 haplotype was not significantly different between AIDS patient with or without toxoplasmic retinochoroiditis ($P = 0.09$).

Table 1

Frequency of human leukocyte antigens (HLA) associated with rapid progression to AIDS in Brazilian AIDS patients presenting with: i) toxoplasmic retinochoroiditis (Group 1); ii) antibody against *T. gondii* but without retinochoroiditis (Group 2); iii) negative serology for *T. gondii* and without retinochoroiditis (Group 3). The frequency of the simultaneous presence of at least 3 antigens of the HLA-A11-Cw4-B35-DR1-DQ1 haplotype associated with rapid progression to AIDS is also shown

HLA	Group 1 (n = 25)	Group 2 (n = 43)	Group 3 (n = 30)	Group Comparisons (P values)		
				1 X 2	1 X 3	2 X 3
HLA-A11*	2 (8%)	2 (5%)	2 (7%)	NS	NS	NS
HLA-B35*	12 (48%)	6 (14%)	14 (47%)	0.0038	NS	0.003
HLA-DR1*	5 (20%)	10 (23%)	3 (10%)	NS	NS	NS
HLA-DQ1*	17 (68%)	30 (70%)	16 (53%)	NS	NS	NS
At least 3 components of haplotype	4 (16%)	0 (0%)	1 (3%)	0.01	NS	NS

*Frequencies of these antigens in the healthy Brazilian population (HLA-A11 = 12%; B35 = 47%; DR1 = 15%; DQ1 = 87%).

NS (non-significant comparisons).

With respect to HLA markers, the HLA-B35 was the most frequent antigen among AIDS patients with toxoplasmic retinochoroiditis in comparison to AIDS patients exhibiting positive anti-*T. gondii* circulating antibodies but without retinochoroiditis ($P = 0.0038$, $RR = 2.56$, $EF = 0.29$).

Considering the patients without retinochoroiditis, the frequency of HLA-B35 antigen was higher in those with negative serology for *T. gondii* ($P = 0.003$, $RR = 0.42$, $EF = 28.40$ (Table 1)).

4. Discussion

Several reports studying the role of HLA specificities in AIDS susceptibility have been published [5], and the haplotypes encompassing HLA-B35 antigens are consistently associated with rapid progression to AIDS in several populations [20]. In a previous study, conducted by us, the frequency of HLA-DQ2 and HLA-B35 antigens were significantly increased in Brazilian AIDS patients presenting with cytomegalovirus retinitis [15].

Few studies have been conducted evaluating the association between HLA alleles and toxoplasmic infection among AIDS patients [21], and there are no reports evaluating HLA associations with ocular disease caused by *T. gondii* infection among AIDS patients. A previous study, evaluating HLA-A and HLA-B antigens revealed no association with in patients presenting toxoplasmic retinochoroiditis but without AIDS [22].

This is the first study evaluating the frequency of HLA markers in Brazilian AIDS patients with ocular toxoplasmic disease, and suggest that HLA markers associated with rapid progression to AIDS (HLA-A11-Cw4-B35-DR1-DQ1) were also associated with toxoplasmic retinochoroiditis, in particular the HLA-B35 antigen.

In Brazil, the frequency of *Toxoplasma gondii* infection is high, and in patients with AIDS reaches values as

high as 71% [10]. Then, HLA markers associated with rapid progression to AIDS may represent additional risk factors for the development of retinochoroiditis in the presence of *T. gondii* infection, suggesting that these patients might be more susceptible to this ocular disease when compared with patients without these markers. The fact that AIDS patients without anti-*T. gondii* antibodies presented increased HLA-B35 antigen frequency may indicate that these individuals are also susceptible to toxoplasmic ocular disease if exposed to *T. gondii*.

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