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Toxoplasmic encephalitis: role of Human Leucocyte Antigens/alleles associated with rapid progression to Acquired Immunodeficiency Syndrome



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ABSTRACT

Background/aims: The frequency of Human Leucocyte Antigens/alleles associated with rapid progression from Human Immunodeficiency Virus infection to Acquired Immunodeficiency Syndrome was evaluated in Brazilian patients with Acquired Immunodeficiency Syndrome with and without Toxoplasmic Encephalitis.

Methods: 114 patients with Acquired Immunodeficiency Syndrome (41 with Toxoplasmic Encephalitis, 43 with anti-*Toxoplasma gondii* antibodies, without Toxoplasmic Encephalitis, and 30 without anti-*Toxoplasma gondii* antibodies circulating and without Toxoplasmic Encephalitis) were studied.

Results: Human Leucocyte Antigens/alleles associated with rapid progression to Acquired Immunodeficiency Syndrome, particularly HLA-B35, -DR3, and -DR1 allele group, were significantly less represented in patients with Toxoplasmic Encephalitis and Acquired Immunodeficiency Syndrome.

Conclusion: The presence of these Human Leucocyte Antigens/Alleles that predispose to Acquired Immunodeficiency Syndrome progression was associated with resistance to Toxoplasmic Encephalitis among Human Immunodeficiency Virus-1 carriers.

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Introduction

Human leukocyte antigens (HLA) genes have been reported to be associated with increased susceptibility to the development of specific disease or with progression to AIDS outcomes.¹⁻³ The progression from human immunodeficiency virus (HIV) infection to AIDS has been strongly associated with HLA-A1-Cw7-B8-DR3-DQ2 and HLA-A11-Cw4-B35-DR1-DQ1 haplotypes, conferring a high risk of rapid progression to AIDS.⁴⁻⁷ It has been assumed that associations between progression to AIDS and particular HLA alleles reflect differential antigen presentation by classes I or II molecules exhibiting particular motifs in the peptide binding groove.⁸ For example, the most harmful effects of HLA-B*35 are seen with the molecules encoded by the HLA-B*35:02 and B*35:03 alleles, which have proline at anchor position 2 of their loaded peptide and a non-tyrosine residue at position 9.⁹ For instance, the HLA-B*35:01 molecule containing tyrosine at position 9 does not have any substantial effect on disease prognosis. While both HLA-B*35 subtypes can equally induce a cytotoxic T lymphocyte (CTL) response, viral load was cleared less effectively by non-tyrosine-containing HLA-B*35:02 and B*35:03 molecules compared with HLA-B*35:01.¹⁰ It may, therefore, be possible that altered epitope recognition by HLA-B*35:02 and B*35:03 will induce CTL that may not specifically function against HIV-1-infected cells.¹¹

Toxoplasma gondii infection is widespread in humans, with estimated infection rates ranging from 50% to 80% of the general population in South America.¹² In some areas of Southern Brazil, the prevalence of antibodies against *T. gondii* may be as high as 98%.^{13,14} Toxoplasmosis in the immunocompromised host is most probably due to reactivation of a previous latent infection and can be life-threatening.¹⁵ Encephalitis is the most important manifestation of toxoplasmosis in immunosuppressed patients as it causes severe damage and death.¹⁶ It is estimated that in countries with a high prevalence of *T. gondii*, toxoplasmic encephalitis is the most common cerebral lesion in HIV patients.¹⁷

Few studies reported an association between HLA markers and toxoplasmic encephalitis in AIDS patients.¹⁸⁻²¹ We have previously reported that susceptibility to toxoplasmic retinochoroiditis was associated with HLA alleles related with rapid progression to AIDS,²² and the availability of genetic markers for other AIDS severe complications may discriminate patients with poor prognosis. To further explore whether HLA markers associated with rapid progression to AIDS could also be associated with the development of toxoplasmic encephalitis, we evaluated these markers in Brazilian AIDS patients with or without toxoplasmic encephalitis.

Material and methods

Patients

The study was conducted on 114 adult HIV-infected patients (81 males) aged 21-59 years (median=33) presenting AIDS, diagnosed 1-108 months (median=22) before inclusion in this study. Forty-one patients experienced toxoplasmic

encephalitis, diagnosed clinically and by brain computerized tomography and by the presence of antibody against *T. gondii* (Group 1). Two additional AIDS patient groups without toxoplasmic encephalitis were studied; i.e., a group of 43 patients with positive anti-*T. gondii* antibodies but without toxoplasmic encephalitis (Group 2), and 30 patients with neither anti-*T. gondii* antibodies nor toxoplasmic encephalitis (Group 3). Patients were selected from the Acquired Immunodeficiency Outpatient Clinic at the University Hospital of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil. A total of 161 healthy bone marrow donors from the University Hospital of Faculty of Medicine of Ribeirão Preto with no known infectious, chronic, or autoimmune disorders were also studied.

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).

Anti-*T. gondii* antibodies

The search for anti-*T. gondii* antibodies in serum was performed by indirect immunofluorescence by the method of Camargo²³ using an anti-human IgG fluorescent conjugate (Bio-Mériéux). Serum samples with >1/16 titers were considered to be positive.

HLA typing

HLA class I antigens expressed on the surface of peripheral blood lymphomononuclear cells were typed using a microlymphocytotoxicity assay.²⁴ DNA was obtained from peripheral blood mononuclear cells using a salting out procedure. HLA class II allele typing was performed using commercial kits (One Lambda, Canoga Park, CA), as previously described.²⁵

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^{4,26} in many ethnic groups, these markers were considered for analysis in the present study.

Statistical analysis

HLA antigen and HLA allele group frequencies were calculated by direct counting. The strength of the association between toxoplasmic encephalitis and HLA specificities was evaluated calculating the relative risk (RR) and Odds Ratio (OR). The Fisher's exact test was used for comparisons, and it was considered to be significant at $p < 0.05$.

Table 1 – Frequency of human leukocyte antigens (HLA) associated with rapid progression to AIDS in Brazilian AIDS patients presenting with: (i) toxoplasmic encephalitis (Group 1); (ii) antibody against *T. gondii* but without toxoplasmic encephalitis (Group 2); and (iii) negative serology for *T. gondii* and without toxoplasmic encephalitis (Group 3). The frequencies of HLA among healthy controls are also shown.

HLA	Group 1 (n=41)	Group 2 (n=43)	Group 3 (n=30)	Healthy controls (n=161)	Group Comparisons (p-values)		
					1 × 2	1 × 3	2 × 3
HLA-A11	4 (10%)	2 (5%)	2 (7%)	19 (12%)	NS	NS	NS
HLA-B35	4 (10%)	6 (14%)	14 (47%)	76 (47%) (p=0.0003 × G1)	NS	0.0007	0.0031
HLA-DR1	5 (12%)	10 (23%)	3 (10%)	24 (15%)	NS	NS	NS
HLA-DQ1	17 (42%)	36 (84%)	16 (53%)	140 (87%) (p=0.0001 × G1)	0.0001	NS	0.008

NS, non-significant comparisons; × G1, comparisons to Group 1.

Results

HLA profile according to the presence of toxoplasmic encephalitis

The frequency of HLA-B35 antigen was significantly decreased among AIDS patients presenting toxoplasmic encephalitis (Group 1) in comparison to AIDS patients with neither anti-*T. gondii* antibodies nor toxoplasmic encephalitis (Group 3) ($p=0.0007$), presenting a RR=0.20 and an OD=0.12 (Table 1). Similar results were observed when the group of toxoplasmic encephalitis AIDS patients (Group 1) were compared with healthy controls ($p=0.0003$) and a RR=0.20 and an OD=0.12 (Table 1). When the HLA-B35 antigen frequency was compared between AIDS patients without toxoplasmic encephalitis (Group 2 and Group 3) its frequency was significantly decreased among AIDS patients presenting with anti-*T. gondii* antibodies (Group 2) ($p=0.0031$), with a RR=0.29 and an OD=0.18 (Table 1). However, the comparison of the frequency of HLA-B35 between patients with positive anti-*T. gondii* antibodies, with and without toxoplasmic encephalitis (Group 1 and Group 2), showed no significant difference (Table 1).

On the other hand, the frequency of HLA-DQB1*01 allele group was significantly decreased among AIDS patient presenting toxoplasmic encephalitis (Group 1) in comparison to AIDS patient presenting anti-*T. gondii* antibodies but without toxoplasmic encephalitis (Group 2) ($p=0.0001$), and with a RR=0.49 and an OD=0.13 (Table 1). Similar results were observed when the AIDS group with toxoplasmic encephalitis (Group 1) were compared with healthy controls ($p=0.0001$), with a RR=0.47 and an OD=0.10 (Table 1). When the HLA-DQB1*01 allele group frequency was compared between AIDS patients without toxoplasmic encephalitis (Group 2 and Group 3) its frequency was significantly decreased among those without anti-*T. gondii* antibodies (Group 3) ($p=0.008$), conferring a RR=1.57 and an OD=4.50 (Table 1).

The frequencies of other HLA markers associated with rapid progression to AIDS were closely similar among AIDS patients and healthy controls.

Discussion

Several reports examining the role of HLA antigens/alleles in AIDS susceptibility have been published⁵ and the haplotypes encompassing HLA-B35 antigens were consistently associated with rapid progression to AIDS in several populations.²⁷ HLA-B*35 alleles have been classified into two groups based on the residue at pocket 9 (P9) of the peptide binding groove. The PY group binds mainly to a tyrosine (Y) at P9, whereas the Px group has a preference for smaller hydrophobic residues such as leucine, methionine, or valine, and does not bind to tyrosine at P9.⁹ HLA-B*35:02 and B*35:03 alleles, which code part of the Px group, have been associated with an especially poor HIV disease outcome.¹⁰ The possible mechanisms for this association remain unknown, but it has been suggested that the greater ability of the HLA-B*35:01 (PY) molecule to present HIV peptides (Gag) compared to HLA-B*35:02/35:03 molecules is a key difference affecting HIV disease outcome.²⁸ Furthermore, HLA-B35:16 another member of the PY group, was the worst HIV-peptide binding molecule among all B35 subtypes and was associated with the highest viral load. Therefore, the detrimental effect of HLA-B35 is unlikely to be related exclusively to PY/Px groups. Other factors, such as the fine specificity of the HIV peptides presented by different B35 molecules, may play a role, affecting the nature of the CTL response.²⁹

Few studies have focused on the evaluation of HLA antigens/alleles in toxoplasmic infection among AIDS patients. Concerning cerebral toxoplasmosis, the study conducted in Caucasian North American patients with AIDS have reported an association of the HLA-DQ3 antigen with susceptibility, and HLA-DQ1 antigen with resistance to toxoplasmic encephalitis.^{19,20} In the present study, we found a similar association; i.e., HLA-DQ1 conferring resistance to toxoplasmic encephalitis in Brazilian AIDS patients. Additionally, Mack et al. studying transgenic mice for DQ human genes, demonstrated that the human DQB1 gene, and to a lesser extent DQ3 gene, confers protection against *T. gondii*, corroborating the idea that certain DQB1 genes are associated with protection against this pathogenic protozoan.

This is the first study evaluating the frequency of HLA markers in Brazilian AIDS patients, a mixed population,

presenting with cerebral toxoplasmic disease. The findings suggest that the frequency of HLA markers associated with rapid progression to AIDS, in particular the HLA-DQB1*01 allele group and HLA-B35 antigen, were less represented among toxoplasmic encephalitis AIDS patients. The frequency of *T. gondii* infection in Brazilian AIDS patients can be as high as 98%.¹³ Therefore, the presence of these HLA markers may confer resistance to the development of toxoplasmic encephalitis, which are different from those markers associated with rapid progression to AIDS.

In conclusion, AIDS patients presenting HLA-DQB1*01 allele group appear to be resistant to the development of toxoplasmic encephalitis, since the frequency of this allele was lower in AIDS patients presenting encephalitis in relation to AIDS patients presenting only the infection (anti-*T. gondii* antibodies). The present study suggests that HLA-DQB1 typing, better than the HLA-B, may help on decisions regarding toxoplasmosis prophylaxis. Further studies will be required to determine if genetic control of susceptibility to toxoplasmic encephalitis is similar in AIDS patients of other ethnicities.

Conflicts of interest

The authors declare no conflicts of interest.

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