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ASSOCIATION OF HLA-DQ AND HLA-DR ALLELES WITH SUSCEPTIBILITY OR RESISTANCE TO HIV-1 INFECTION AMONG THE POPULATION OF CHACO PROVINCE, ARGENTINA

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Abstract The pathogenesis of infections clearly involves immunoregulatory host factors and products of Major Histocompatibility Complex (MHC) genes class II which present antigenic peptides to the T-cell receptor on CD4+ cells which in turn increase the production of specific antibodies and cytotoxic T lymphocytes. The objective of this study was to determine the frequency of the different alleles of HLA class II DQ and DR in HIV-1 infected patients of Caucasians with Guaraní and Toba genetic backgrounds in an effort to determine the prevalence of certain alleles which could signify a factor of susceptibility to or protection against HIV-1 infection. A total of 54 HIV-1 positive patients and 46 healthy control subjects participated in the HLA-DQB1 study while 54 HIV-1 (+) patients and 57 healthy controls were analyzed for HLA-DRB1. Both HLA-DQB1 and HLA-DRB1 genotyping were performed using PCR and sequence-specific reverse hybridization oligonucleotide probe and analyzed with the LiPA Key Typing System and LiPA software. HLA-DQB1*0203(P=0.041) and DRB1*01(*P*=0.05) exhibited a decreased frequency in HIV-1 (+) patients while HLA-DRB1*13 (*P*=0.017) was observed more frequently. Several studies have reported different findings, depending on the populations analyzed. Our data show that there are HLA class II alleles associated with susceptibility or resistance to HIV-1 infection and that these differ among ethnic groups. We believe that our results differ from the other Caucasians populations due to the ethnic variability of Chaco inhabitants resulting from mixing between Caucasians and South American natives (Guaraníes and Tobas)

Key words: HIV-1, MHC, HLA, gene frequency, cytotoxic lymphocytes

Resumen ***Asociación de los alelos HLA-DQ y HLA-DR con susceptibilidad y/o resistencia a la infección por HIV-1 en la población del Chaco, Argentina.*** Los linfocitos citotóxicos (CTL) juegan un rol preponderante en el control de las enfermedades virales cuya eficacia depende en gran medida de la interacción con los péptidos presentados en el contexto de moléculas HLA Clase I. Las moléculas HLA Clase II son los elementos de restricción para los LT CD4+, responsables de montar una respuesta humoral y de cooperar con la acción citolítica. El objetivo de este estudio fue determinar la frecuencia de los diferentes alelos de HLA clase II DQ y DR en pacientes infectados por HIV-1 en la Provincia del Chaco con el fin de investigar si la presencia de ciertos alelos puede conferir susceptibilidad o protección a la infección por HIV. Las muestras fueron tipificadas por biología molecular, con la técnica de hibridación reversa con oligonucleótidos secuencia específica (INNO-LiPA) y analizados con LiPA software. Se analizaron 54 muestras de pacientes HIV-1 (+) para HLA-DQ y HLA-DR, 46 controles sanos para HLA-DQ y 57 controles sanos para HLA-DR. Se encontró una menor frecuencia de los alelos HLA-DQB1*0203 (*P*=0.041) y DRB1*01 (*P*=0.05) en HIV (+) El alelo DRB1*13 (*P*=0.017) fue observado más frecuentemente en HIV (+). Nuestros datos indican alelos HLA clase II que podrían estar asociados con susceptibilidad y resistencia a la infección por HIV-1 y que éstos difieren en los distintos grupos étnicos. Pensamos que las diferencias halladas con otras poblaciones caucásicas se deban a que la población chaqueña de origen caucásico tiene una fuerte mezcla con guaraní y toba.

Palabras claves: HIV-1, MHC, HLA, frecuencia génica, linfocitos citotóxicos

The pathogenesis of infections clearly involves immunoregulatory host factors and products of Major Histocompatibility Complex (MHC) genes class II, which present antigenic peptides to the T cell receptor on CD4+ cells, which in turn increase the production of specific antibodies and cytotoxic T lymphocytes. MHC class I genes encode molecules that present host –or microbe– derived antigenic peptides to cytotoxic CD8 cells, which can attack and eliminate the antigen-presenting cells. The latter peptides are generated in the cytosol, transported across the endoplasmic reticulum by the protein products of TAP genes and assembled with class I heavy and light chains for presentation at the cell surface[1-3].

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Certain HLA antigens have been reported to be more frequent in patients with the acquired immunodeficiency syndrome (AIDS). In addition, different HLA antigens seem to influence the variability of disease progression in patients from different risk groups and ethnic background.

However, it has been difficult to establish whether specific HLA alleles are strong or consistent determinants of HIV-1 disease progression in populations. Inconsistencies have arisen from differences in ethnicity, small sample sizes in early studies, investigative designs, laboratory techniques, and statistical methods, as well as extreme polymorphism in HLA[4-6]

Roe et al[7] found DQB1*0603 associated with HIV-1 infection in Caucasians but not African Americans. DQB1*03032 frequencies indicate a positive association with protection in Caucasians but not African Americans. DQB*0201 was observed more frequently in HIV-1(+) than in HIV-1(-) African Americans, suggesting a positive association with HIV-1 infection in this ethnic group. HLA-DRB*04 exhibited a positive association with HIV-1 infection in Caucasians.

Rohowsky-Kochan et al[8] report prevalence significantly higher of DQ7 in HIV-1(+) compared to seronegative Black and Hispanic individuals. The frequency of DQ4 was significantly elevated in the Black seronegatives.

The objective of this study was to determine the frequency of the different HLA class II DQ and DR alleles in HIV-1 infected patients in Chaco Province, Argentina, in an effort to detect the presence of certain alleles that could be a factor of susceptibility to or protection against HIV-1 infection.

Material and Methods

A total of 54 HIV-1(+) patients and 46 healthy individuals were analyzed for HLA-DQB1and 54 HIV-1(+) patients and 57 healthy subjects for HLA-DRB1. All of them belonged to Cau-casians with Guaraní and Toba genetic backgrounds living in Chaco Province.

The DNA samples obtained from peripheral blood were prepared by the salting-out method and then resuspended in distilled water. The HLA-DQB1 and HLA-DRB1 genotyping was performed using PCR and sequence-specific oligonucleotide probe reverse hybridization (LiPA Key Typing System) and analyzed with the LiPA software.

Statistical analysis: The objective of the analysis was to examine the association between allele prevalence and HIV-1 seroposivity. For each allele, the proportion of HIV-1 positive patients and control subjects was compared using the gene frequency (GF), which was calculated as GF=1- $\sqrt{1\text{-AF}}$; AF (antigen frequency) was calculated by using Haldane's method. The degree of association between an HLA allele was expressed as the odds ratio (OR), which was calculated according to Woolf's formula, and *P* was determined by χ^2 analysis or, when appropriate, by Fisher's exact test or Yates correction. $P < 0.05$ was considered significant[9].

Results

The gene frequency of HLA class II alleles among positive patients was compared with healthy controls, 54 HIV-1

TABLE 1.– *Distribution of HLA-DQ alleles among 54 human immunodeficiency virus (HIV)-positive patients and 46 control subjects*

	HIV (+)		Controls			
Allele	n	GF(%)	N	GF(%)	*P**	OR
DQB1*0201/0202	19	19.5	17	20.6		
DQB1*0203	2	1.9	8	9.1	0.041*	0.18
DQB1*03011/03012/ 0309	20	20.6	16	19.2		
DQB1*0302/0307	15	15.0	12	14.0		
DDQB1*03032/03033/0306	4	3.8	7	7.9		
DQB1*0304	2	1.9	3	3.3		
DQB1*0402	9	8.7	2	2.2	0.10**	4.40
DQB1*05011	9	8.7	8	9.1		
DQB1*0502	0	0	1	1.1		
DQB1*05031	1	0.9	2	2.2		
DQB1*06011/06013	3	2.8	2	2.2		
DQB1*0602/0611	6	5.7	5	5.6		
DQB1*0603/0614	10	9.7	4	4.4		
DQB1*0604	5	4.7	4	4.4		
DQB1*0607	2	1.9	0	0		
DQB1*0613	0	0	1	1.1		

GF, gene frequency; OR, odds ratio.
**Fisher's exact 2-tailed test; ** Yates correction.*

TABLE 2.– *Distribution of HLA-DR alleles among 54 human immunodeficiency virus (HIV)-positive patients and 57 control subjects*

	HIV (+)		Controls			
Allele	n	GF(%)	n	GF(%)	*P*	OR
DRB1*01	7	6.7	17	16.2	0.050*	0.35
DRB1*03	9	8.7	8	7.3		
DRB1*04	31	34.7	27	27.5		
DRB1*07	14	13.9	13	12.1		
DRB1*08	5	4.7	6	5.4		
DRB1*09	0	0	3	2.7		
DRB1*10	2	1.9	2	1.8		
DRB1*11	4	3.8	9	8.2		
DRB1*12	1	0.9	0	0		
DRB1*13	19	19.5	8	7.3	0.017*	3.33
DRB1*14	6	5.7	5	4.5		
DRB1*15	6	5.7	10	9.2		
DRB1*16	4	3.8	6	5.4		

GF, gene frequency; OR, odds ratio.
** Yates correction.*

positive patients and 46 healthy individuals were analyzed for HLA-DQB1 and were studied 54 HIV-1 positive patients and 57 healthy individuals for HLA-DRB1 of Caucasians with Guaraní and Toba genetic backgrounds living in Chaco Province, Argentina.

Tables 1 and 2 show the distribution of DQ and DR alleles in HIV-1(+) patients and control subjects, ORs, *P* and χ^2 values for alleles that present significant difference with respect to the healthy population. The OR for DQB1*203(0.18) was decreased with *P* significant P=0.041 whereas the OR DQB1*402(4.40) was elevated, without *P* statistically significant (*P*=0.10).

With regard to the DR locus, allele DRB1* 13 occurred more frequently among HIV-positive patients than among control subjects (OR =3.33; *P*=0.017) and allele DRB1*01 (OR=0.35; *P*=0.050) occurred less frequently in the same population.

Discussion

The increased or decreased frequency of certain alleles in HIV-1(+) patients, compared with control subjects, would suggest that they are a factor in susceptibility to or protection against the viral infection. Several studies reported different findings, depending on the populations analyzed. We determined alleles of the HLA class II (DQ and DR) in order to understand if these genetic factors are associated with susceptibility or resistance to HIV-1 infection among a population in Chaco Province of Northeast Argentina (Caucasians with Guaraní and Toba genetic backgrounds).

We observed that DRB1*13(*P*=0.017) occurred more frequently among HIV-1(+) patients than among controls suggesting that it plays a role in susceptibility to HIV-1 infection**.** Winchester et al[10] reported the transmission of HIV-1 from an infected woman to her offspring during gestation and delivery was found to be influenced by the infant's major histocompatibility complex class II DRB1 alleles. One or more DR13 alleles, including DRB1*1301, 1302, and 1303, were found in 31.7% of seroreverting infants and 15.2% of those becoming HIV-infected (OR=2.6 *P*=0.048). This association was influenced by ethnicity, being seen more strongly among the 80 Black and Hispanic children (OR=4,3; *P*=0.023), with the most pronounced effect among Black infants where 7 of 24 seroreverters inherited these alleles as compared to none among 12 HIV-infected infants (OR=12.3; *P*=0.037). The previously recognized association of DR13 alleles with some situations of long-term nonprogression of HIV suggests that similar mechanisms may regulate both the occurrence of infection and disease progression after infection.

Upon examining for residual associations, only the DR2 allele DRB1*1501 was associated with seroreversions in Caucasoid infants (OR=24; *P*=0.004). Among Caucasoid the DRB1*03011 allele was positively associated with the occurrence of HIV infection (*P*=0,03)[10].

We noted a decreased frequency in the alleles DQB1*203(*P*=0.041) and DRB1*01(*P*=0.05) among HIV-1 (+) compared with control subjects which would suggest that they are a factor in protection against the viral infection. MacDonald[11] observed that resistance to HIV-1 infection was independently associated with HLA-DRB1*01(IRR, 0.22; 95% CI; 0.06-0.60; *p*=0.0003) which suggests that anti-HIV-1 class II restricted CD4 effector mechanisms may play an important role in protecting against viral challenge. On the other hand, Roe et al[7] found DQB1*0603 and DRB1*04 in a positive association with HIV-1 infection in Caucasians but not in African Americans, while DQB1*0201 was observed more frequently in HIV-1 positive than HIV-1 negative African Americans, suggesting protection against HIV. DQB1*03032 frequencies indicating a positive association with protection from HIV-1 infection in Caucasians.

Other authors observed that HLA markers associated with possible protection from infection for African Americans were Cw4 and DRw6, whereas Caucasians expressed none[12].

Just et al[13] studying the role of host genotype in pediatric infection with HIV-1 and progression to AIDS, described the presence of DQB1*0604 associated with increased risk of HIV infection.

In Italy, a higher frequency of HLA-A2 has been reported in HIV-1(+) patients, compared with HIV-1 seronegatives, at-risk patients (patients with hemophilia), whereas HLA-B52 and DR4 were decreased in HIV-1 positive patients[14].

Other authors informed that the frequency of DQ4 was significantly elevated in the Black seronegatives and report prevalence significantly higher of DQ7 in HIV-1 (+) compared to seronegatives Black and Hispanic individuals[8]. We observed that OR for DQB1*0402 (4.40) was elevated, without P statistically significant (*P*=0.10).

In conclusion, our data show that there are HLA class alleles associated with susceptibility (DRB1*13) or resistance (DQB1*0203 and DRB1*01) to HIV1(+) infection and that these differences among ethnic groups provide additional evidence that HLA polymorphism may confer a genetic risk or protection for HIV-1 infection. We believe that our results differ from those described in the literature due to the ethnic variability of Chaco inhabitants resulting from the mixing among Caucasians and South American natives (Guaraníes and Tobas).

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Last scene of all that ends this strange eventful history, is second childishness, and mere oblivion, sans teeth, sans eyes, sans taste, sans everything.

La escena final se acerca, poniendo fin a esta peregrina historia tan plena de acontecimientos. Es la segunda infancia y el total olvido: sin dientes, sin ojos, sin gusto, sin nada.

William Shakespeare (1564-1616)

As you like it, 1599