Association of Alport's syndrome with HLA-DR2 antigen in a group of unrelated patients

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Abstract

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A few family studies have evaluated HLA antigens in Alport's syndrome; however, there are no large population studies. In the present report, we studied 40 unrelated white patients with Alport's syndrome seen at the Unit of Renal Transplantation, Faculty of Medicine of Ribeirão Preto, São Paulo, Brazil. HLA-A, -B, -DR and -DQ antigens were typed using a complement-dependent microlymphocytotoxicity assay. A control white population (N = 403) from the same geographical area was also typed for HLA antigens. Although the frequencies of HLA-A and -B antigens of patients were not statistically different from controls, the frequency of HLA-DR2 antigen observed in patients (65%) was significantly increased in relation to controls (26%; $P<0.001$). The relative risk and etiologic fraction for HLA-DR2 antigen were 5.2 and 0.525, respectively. Although few immunological abnormalities have been shown in Alport's syndrome, in this report we emphasize the association of HLA molecules and Alport's syndrome. Besides the well-known inherited molecular defects encoded by type IV collagen genes in Alport's syndrome, the major histocompatibility alleles may be in linkage disequilibrium with these defective collagen genes.

Introduction

Alport's syndrome is a genetic disorder of type IV collagen involving non-homogeneous patterns of inheritance (1). The X-linked form, which has been mapped to the long arm of chromosome X (Xq 22), and the autosomal dominant or recessive varieties which have been located on chromosome 2 are associated with mutations and deletions of the COL4A5 and COL4A3/COL4A4 genes, respectively, which encode α chains

of type IV collagen in glomerular basement membranes (2,3). The disease is clinically characterized by the early onset of hematuria which ultimately progresses to renal failure. Affected patients can also exhibit sensorineural hearing loss and a variety of ocular lesions (4). Although there are no pathognomonic light microscope lesions in kidney biopsies, ultrastructural alterations include variable thickening, thinning, basket-weaving and lamellation of the glomerular basement membrane (GBM). Immunofluores-

cence microscopy findings are usually negative; however, granular deposits of C3 and IgM in mesangium and in glomerular capillary walls may be occasionally seen (2,5). In addition, the GBM of males with Alport's syndrome fails to react with human anti-GBM autoantibodies or monoclonal antibodies against the Goodpasture antigen, which is associated with the α3 chain of type IV collagen (2). Due to the heterogeneous nature of inheritance and to the paucity of immunological abnormalities, only few HLA studies have been conducted on Alport's syndrome, mainly family studies (6-8). Since at least one of the collagen genes (COL11A2) is located within the major histocompatibility complex (MHC) (9), in this study we performed HLA class I and class II antigen typing in a group of unrelated patients with Alport's syndrome.

Patients and Methods

Subjects

We studied 40 patients (23 males) with Alport's syndrome aged 11-53 years (median = 27.5), who had been referred to the Unit of Renal Transplantation at the University Hospital, Faculty of Medicine of Ribeirão Preto, São Paulo, Brazil, to participate in the kidney sharing program of the São Paulo Interior Transplante (SPIT). Clinical, laboratory and/or ultrastructural (kidney biopsies) findings characteristic of Alport's syndrome were observed in all patients. In addition, all patients presented at least one of the following features: hearing loss and/or ophthalmologic abnormalities and/or a family history of Alport's syndrome. Twenty-six patients (16 males) underwent transplantation with kidneys from live or cadaver donors, 11 patients (5 males) were on hemodialysis and 3 patients (2 males) were on continuous ambulatory peritoneal dialysis. A total of 403 white control individuals (212 for HLA-A, -B, and 191 for HLA-DR, -DQ)

from the same geographical area and presumably of similar ethnic background were also typed for HLA antigens.

HLA Antigens

Mononuclear peripheral blood cells were isolated using a Ficoll-Hypaque gradient at a density of 1.077. B-lymphocytes were isolated by adherence to nylon wool (Robbins Scientific, Sunny Valley, CA, USA). HLA typing was performed by a complement-dependent microlymphocytotoxicity assay (10), using commercially available antisera (Pel Freez, Brown Deer, WI, USA; Gen-Trak, Brookfield, WI, USA; Biotest, Dreilich, Germany). Fifty-seven class I (A and B) and 15 class II (DR and DQ) specificities were tested. Complement was obtained from a pool of normal rabbit sera.

Statistical analysis

Comparisons of HLA frequencies between patients and controls were estimated using the two-tailed exact Fisher test, with corrections of P values according to the number of HLA specificities tested. Differences were considered significant at P<0.05. Relative risk (RR), which indicates how many times more often the disease occurs in individuals with the HLA antigen compared to those without it, and etiologic fraction (EF), which defines the attributable risk at the population level, were also calculated (11).

Results

The frequency of HLA-A or -B antigens observed in patients was not significantly different from that observed in control individuals (Table 1). HLA-DR2 specificity was overrepresented in patients with Alport's syndrome compared to controls (P<0.001). Twenty-six of 40 (65%) patients expressed HLA-DR2 antigen, whereas only 50 of 191 (26%) controls presented the same antigen

(Figure 1). The relative risk and etiologic fraction for HLA-DR2 were 5.2 and 0.525, respectively. Statistical analysis of the comparisons of other HLA-DR or -DQ antigens between patients and controls did not show significant differences (Table 1).

Discussion

The characteristics and nature of the genetic defects responsible for ultrastructural glomerular abnormalities seen in Alport's syndrome have been extensively studied in the last 5 years (2,3). Since at least one of the genes responsible for collagen synthesis is located inside the MHC region, and there are few studies focusing on the role of MHC alleles or the products encoded by these genes (HLA molecules) in the susceptibility to the syndrome, in this study we evaluated the possible association between HLA antigens and Alport´s syndrome.

The frequency of HLA-A or -B antigens observed in patients was not significantly different from controls. In a previous report, the study of 6 Mexican Mestizo patients with Alport's syndrome also showed no association with HLA-A or -B antigens (6). In our series, HLA-DR2 antigen was significantly increased, conferring a high relative risk and etiologic fraction. To our knowledge, this is the first report about HLA class I and II antigens in a large group of unrelated patients with Alport's syndrome. Previous family studies have shown the presence of HLA-DR7 in kindreds with Alport's syndrome of German or Jewish origin (7,8). The frequency of HLA-DR7 specificity observed in our patients was not statistically different from that observed in controls (Table 1). The discrepancies between these observations may be attributed to the ethnic differences and to the nature of these studies (related x unrelated patients). HLA-class II antigens are usually associated with diseases presenting underlying immunopathogenetic mechanisms, such as rheumatoid arthritis and insu-

lin-dependent diabetes mellitus (12). Alport's syndrome is not a classical example of a disorder mediated by immunopathogenic mechanisms; however, some immunological abnormalities have been reported. Although most accounts have described nega-

Table 1 - Frequency of HLA class I (A,B) and class II (DR, DQ) antigens in patients with Alport's syndrome (N = 40) and in control individuals (N for class I = 212; N for class II = 191).

The frequency observed in controls is shown within parentheses. *P<0.001 compared to controls (Fisher test).

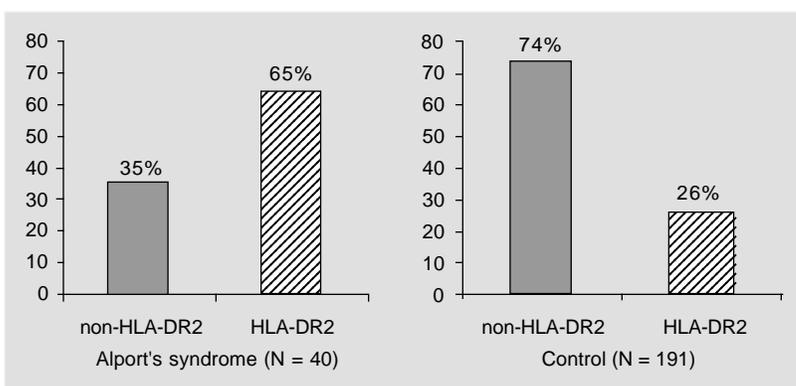| % HLA antigens							
HLA-A		**HLA-B**		**HLA-DR**		**HLA-DQ**	
A1	47(19)	B51	22(19)	DR1	30(29)	DQ1	57(65)
A2	52(54)	B7	20(16)	DR2	65(26)*	DQ2	17(9)
A3	30(16)	B8	5(10)	DR3	17(23)	DQ3	17(77)
A23	20(6)	B44	35(23)	DR4	32(37)		
A24	12(18)	B13	2(5)	DR5	12(24)		
A25	2(6)	B14	7(8)	DR6	12(13)		
A26	2(1)	B15	10(5)	DR7	17(13)		
A11	7(8)	B17	5(6)	DR8	2(2)		
A29	5(6)	B38	5(4)	DR9	2(13)		
A30	5(5)	B39	2(4)	DR10	0(1)		
A31	2(1)	B57	5(6)				
A32	2(1)	B49	20(6)				
A33	2(2)	B50	2(1)				
A28	7(15)	B27	2(3)				
		B35	17(17)				
		B60	5(6)				

Figure 1 - Frequency of HLA-DR2 antigen in patients with Alport's syndrome and controls, compared with other non-HLA-DR2 specificities.

tive immunofluorescence microscopic findings in kidney biopsies from Alport's patients, some studies have shown granular deposits of C3, IgM, IgG and C4 within the mesangium and vascular pole, and along the glomerular capillary walls (2,4,5). Other immunological abnormalities include normal or low levels of serum complement, elevated excretion of C3 polypeptide fragments in urine, presence of antithyroid antibodies, decreased levels of plasma and secretory IgA, and increased frequency of allergic diseases (4,13,14). Whether Alport's syndrome has an autoimmune background, or immunological abnormalities are secondary to inherited defects are questions to be answered.

The occurrence of a posttransplant anti-GBM nephritis in the allograft donated by an HLA-identical sibling (15) corroborates the relevance of the defect in type IV collagen α chain. On the other hand, anti-GBM nephritis occurs in 5 to 10% of males with Alport's syndrome who undergo kidney transplantation. The pathogenic anti-GBM antibody reacts both with normal glomerular and epidermal basement membranes, but not with membranes of most Alport's syndrome patients. These anti-GBM antibodies react with the engrafted GBM in a pattern similar to that observed with Goodpasture sera (15). In this context, 75 to 80% of the patients who develop Goodpasture syndrome have also the HLA-DR2 antigen and the HLA-DRB1*15 allele (16,17). In addition, HLA-B7 antigen together with HLA-DR2 have been associated with higher levels of plasma creatinine, a greater proportion of glomeruli surrounded by crescents, and a worse prognosis (16). None of the transplanted patients in our series developed anti-GBM nephritis, preventing a meaningful calculation of the above associations. Whether Goodpasture syndrome patients and transplanted Alport's patients share immunogenetic susceptibility to the development of anti-GBM nephritis, and whether the association of both syndromes with HLA-DR2 is coincidental are subjects for further investigation.

Finally, MHC genes are located very closely to a gene which is responsible for collagen synthesis, and this gene, COL11A2, is just a few kilobases centromeric to HLA-DP alleles (9). Therefore, it is possible that HLA class II alleles may be in linkage disequilibrium with other genes inside or outside the MHC responsible for collagen synthesis. Further analysis at the molecular level may help us to understand the basis of the particular association between HLA-DR2 specificity or other HLA class II alleles and Alport's syndrome.

Acknowledgments

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