Frequency of *HLA-DQB*06* in Caucasian, African American, and Mexican American patients with a positive direct antiglobulin test

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A reduced frequency of *HLA-DQ6* in patients with a positive direct antiglobulin test (DAT) was previously reported but race was undisclosed. Therefore, we investigated a total of 275 patients (80 Caucasian, 113 African American, and 82 Mexican American) and 518 normal controls (205 Caucasian, 208 African American, and 105 Mexican American). These were typed for class II HLA antigens using molecular techniques. A DAT was performed on each patient's red cells drawn into EDTA using both mouse and rabbit polyspecific reagents. Of 275 patients tested, 73 (27%) had a positive DAT (12 Caucasians, 35 African Americans, and 26 Mexican Americans). We found that 5 (42%) Caucasian patients and 103 (50%) Caucasian controls possessed the *DQB*06* allele ($p = .56$). In the African American group, 15 (43%) patients and 91 controls (44%) were *DQB*06* positive ($p = .92$). Six Mexican American patients (23%) and 21 controls (20%) had the *DQB*06* allele ($p = .72$). This article underscores the need to use race-matched controls when genetic disease associations are sought. ***Immunohematology* 2000; 16:74–7.**

Key Words: human leukocyte antigens (HLA), major histocompatibility complex (MHC), direct antiglobulin test (DAT), autoimmune hemolytic anemia (AIHA)

The major histocompatibility complex (MHC) is a set of genes whose molecular products, the human leukocyte antigens (HLA), play a central role in the control of the immune response. These genes are closely linked and are inherited as a unit or haplotype. The MHC genes, which code for the three classes of HLA antigens, are located on chromosome 6. The class II genes are known as the D region and the individual loci within this area are designated as *DM*, *DO*, *DP*, *DQ*, and *DR*. The *MHC* genes are among the most polymorphic in the human genome, which makes it highly unlikely that any two random individuals will express identical sets of MHC molecules on their cells.

The MHC class II molecules are expressed on B lymphocytes, thymic epithelial cells, and dendritic cells but can also be induced by certain activating factors to appear on such cells as macrophages. Class II molecules are dimers made up of an alpha and a beta chain. Each has both a variable and an invariable region. The primary function of the class II molecules is to present foreign antigenic peptides to CD4 T-helper cells. Those T cells with the appropriate receptor bind to the invariable portion of the class II molecule, become activated, and assist B cells in production of antibody directed against a specific foreign antigen. In this way an immune response is initiated against exogenous antigens such as bacteria and viruses that have invaded "normal" body cells.

Since the early 1990s, PCR amplification of HLA class II genes with single-stranded oligonucleotide detection of polymorphic sites in the DNA fragment has become common. This DNA-based method for molecular typing of all classes of HLA antigens allows for a more precise identification of the unique alleles present in an individual compared with serologic techniques used in the past.

Certain haplotypes of the HLA antigens are associated with particular diseases. For example, the HLA alleles *DR2* and *DR3* are associated with an increased frequency of systemic lupus erythematosus (SLE).[1,2] Associations have been made between HLA-DR and HLA-DQ antigens and other autoimmune diseases, including rheumatoid arthritis, diabetes mellitus, multiple sclerosis, and hemolytic anemia.[3]

Autoimmune hemolytic anemia (AIHA) is often a secondary feature of multisystem rheumatic diseases, particularly SLE. It also may arise in the setting of human immunodeficiency diseases, such as AIDS.[4] AIHA occurs when there is an increased destruction of red blood cells (RBCs) coated with anti-RBC autoantibodies, which are cleared prematurely by the liver and occasionally by the spleen. The bone marrow becomes stressed and cannot adequately compensate for the excessive loss of RBCs. The autoantibodies, which cause anemia, can be classified into two types: Cold reactive autoantibodies react optimally at temperatures less than 37° C and are of the IgM variety. Warm reactive autoantibodies are usually IgG, bind to RBCs best at 37° C, and are the most fre-

quent cause of anemia. In association with other rheumatic diseases, AIHA can be detrimental to the outcome of an illness.

One study of patients with hemolytic anemia found a reduced frequency of the class II *HLA-DQ6* allele.[5] In this study the hemolysis was accompanied by a positive DAT. The study concluded that DAT-positive individuals were less likely to possess the *DQB*06* allele and people who did possess the allele may be protected from clinically relevant RBC autoantibodies. The study design for the research included 31 samples from patients with a positive DAT, most of whom displayed evidence of clinical hemolysis. Thirteen of those patients had an associated disease, ten had hemolysis only, and eight were DAT-positive normal blood donors with no signs of hemolysis. The control group consisted of 85 DAT-negative cadaver organ donors. The race-ethnicity of the patients and controls was not disclosed but was said to include minorities. The patients and controls were typed for *HLA-DR* and *HLA-DQ* class II alleles. Results showed that only 13 percent of the DAT-positive patients possessed the *DQB*06* allele, compared with 53 percent of the DAT-negative normal controls ($p = 0.059$, RR = 0.23).

We examined a much larger group of patients and controls, giving special consideration to race-ethnicity because it has been proven that different racial-ethnic groups have a propensity for certain genetic haplotypes.[6] Our purpose was to determine *HLA-DQB* gene frequency in three populations and to ascertain whether an association between the *HLA-DQB*06* allele and hemolytic anemia with a positive DAT existed.

Materials and Methods

Test subjects

Individuals recruited for this study were approved by the Committee for the Protection of Human Subjects. Three racial-ethnic groups were chosen for our cohort: Caucasian, African American, and Mexican American, representing 275 patients and 518 normal controls. Of the Caucasian group, 80 were patients and 205 were controls. The African American group had 113 patients and 208 controls, whereas the Mexican American group contained 82 patients and 105 controls. Each patient was diagnosed as having either SLE (197) or AIDS (78), two diseases in which a positive DAT is a frequent finding.[7] Approximately 10 percent of the SLE patients had clinical symptoms of hemolysis (G. Alarcon, personal communication).

Direct antiglobulin testing (DAT)

DATs were performed using the standard tube agglutination method. A 3 to 5% washed cell suspension was made from EDTA blood samples for each patient and control tested. One drop of the cell suspension and 2 drops of rabbit or murine polyspecific anti-human globulin (Gamma Biologicals, Houston, TX) were mixed, then centrifuged for 15 seconds at 3000 rpm. Agglutination was examined macroscopically with the aid of an agglutination viewer, and results were graded w+ to 4+.[8] Positive DATs were characterized using monospecific murine and rabbit anti-IgG reagents and a murine anti-C3b-C3d reagent (Gamma Biologicals). Because we have noted a number of falsely positive reactions when using rabbit polyspecific antiglobulin reagents (unpublished data), only those samples that were positive for IgG were included in this study.

HLA typing

Genomic DNA was isolated from peripheral blood leukocytes by proteinase K digestion followed by phenolchloroform extraction.[9] *HLA* class II genotyping of *HLA-DRB1*, *DQA1*, and *DQB1* alleles was performed by oligotyping as previously described, with modifications added to type for the currently recognized DRB1 specifcities.[10,11] High resolution HLA-DRBl typing was carried out using a two-step method. First, generic DRB typing was performed to distinguish the *DR1, DR2, DR3/6/11, DR 8/12*, and *DR4* allelic groups as well as *DRB1*0701, 0901, 1001*; *DRB3*0101,*02/03*; and *DRB4*0101*. Subsequently, group-specific amplification of *HLA-DR1, DR2, DR3/6/8/11/12*, and *DR4* alleles was carried out using the appropriate primers for high resolution typing with 18-mer oligonucleotide probes known to determine all alleles at the respective loci.

Statistical analysis

Statistical analysis for determining the frequency of the *DQB*06* allele in both patients and race-ethnicity–matched controls was performed using a two-by-two contingency table (chi-square analysis) with the EPI-INFO statistical program. Statistical significance was determined by Fisher's exact probability test.

Results

It was found that 73 of the 275 patients (27%) were DAT-positive. Twelve of the 80 patients in the Caucasian group (15%), 35 of the 113 African American patients (31%), and 26 of the 82 Mexican American patients

(32%) had positive DAT results. All 518 members of the control group were DAT-negative (Table 1).

Table 1. Total patients and controls grouped by race-ethnicity

	No. of patients	Patients with a positive DAT*	Controls with a negative DAT
Caucasian	80	12 (15%)	205
African American	113	35 (31%)	208
Mexican American	82	26 (32%)	105
Totals	275	73 (27%)	518

*Direct antiglobulin test

Controls and DAT-positive patients were then tested for the presence of the *HLA-DQB*06* allele using the DNA-based method for class II HLA typing. Of those patients with a positive DAT, 42 percent of Caucasians possessed the *DQB*06* allele as did 50 percent of the Caucasian DAT-negative controls. In the African American group, 43 percent of DAT-positive patients displayed the *DQB*06* allele as well as 44 percent of the African American controls. Twenty-three percent of the DAT-positive Mexican American patients tested positive for the *DQB*06* allele compared with 20 percent of the Mexican American controls (Table 2). A chi-square analysis of these data revealed that differences were not statistically significant ($p > .5$).

Table 2. Frequency of *HLA-DQB*06* in DAT-positive patients and DAT-negative controls

	Patients with a positive DAT*	Controls with a negative DAT	*p*-value
Caucasian	5 (42%)	103 (50%)	.56
African American	15 (43%)	91 (44%)	.92
Mexican American	6 (23%)	21 (20%)	.72

*Direct antiglobulin test

Discussion

There appeared to be racial-ethnic differences in the DAT results that have not been reported previously. Twice as many African American and Mexican American patients (31% and 32%, respectively) had positive DATs than did the Caucasian patients (15%). A possible explanation for this is that African American and Hispanic SLE patients tend to have more severe disease than do Caucasian SLE patients.[12] In our study, 72 African American and 71 Mexican American patients were diagnosed with SLE. The high frequency of positive DATs in those ethnic groups may be due to high levels of immune complexes that may accompany the disease.

There is a generalized reduction in the Mexican American population for the *DQB*06* allele. Likewise, there appears to be no association between the *DQB*06* gene and a positive DAT. Conclusions from a previous investigation may have been skewed due to the inappropriate matching of controls used in that study group. Thus, this article underscores the need to use race-ethinicity-matched controls when investigating genetic associations with diseases.

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