Analysis of HLA-DP DNA typing on the Antiphospholipid Syndrome

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Introduction
It is well known that fetal losses, preeclampsia, and IUGR are major complications for pregnant women with systemic lupus erythematosus 1). And significantly high positive rate of antinuclear antibodies and anticardiolipin antibody has been reported in patients with spontaneous abortion. Recently, Antiphospholipid Syndrome (APA-S) is recognised as a new autoimmune disease introduced by Harris et al.2) This disease is characterized by venous or arterial thrombosis, recurrent abortions, thrombocytopenia in clinical presentation. It is well known that some autoimmune diseases have associations with certain HLA antigens. To clarify the association of HLA class II and APA-S, we adopted the PCR-RFLP method for genotyping of the HLA-DPBL genes.

Materials and Methods
1. Materials
Thirty nine women with positive APA who had suffered from recurrent abortions were analyzed in this study (APA-S group). All of the subjects had experienced two or more spontaneous abortions, and none of them indicated the presence of other considerable reasons of abortions. Fourty three unrelated healthy individuals were included in the control group.

2. Method for measurement of APA
Antiphospholipid titer was determined by ELISA against cardiolipin and phosphatidyl serine. Optical density above 3 standard deviations of normal individuals was considered to be positive.

3. Method for DNA samples and analysis
The DNA was extracted with phenol and chloroform-isomyalcohol, precipitated with ethanol and solubilized in 10mM Tris-HCl/1mM EDTA, pH8.0.
Genomic DNA (1 fÊg) was amplified by the PCR procedure with 2.5 units of the Taq DNA polymerase. The reaction mixture and distilled water for a total volume 100 fÊl in a 1.5 ml eppendorf tube with 50 fÊl of mineral oil to prevent evaporation was subjected to 30 cycle of 1 min at 94°C, 1 min at 62°C, and 2 min at 72°C by a thermal cycler. A two hundred and ninety nine base pair fragment from the second exon of the HLA-DPB1 gene was amplified by using 1 fÊM each of the PCR primers DPB101N and DPB201 according to the methods described by Ota et al.3). After amplification, aliquots (6 fÊl) of the reaction mixture were digested with restriction endonucleases (5units) 3h adding the appropriate incubation buffer (1 fÊl) and distilled water up to a final volume of 10 fÊl. Bsp 1286I, Fok I, DdeI,
BsaI, BssHII, Cfr13I, RsaI, EcoNI, AvaII were used to perform single digests on the amplified DPB1 genes. The samples were subjected to electrophoresis in a 12% polyacrylamide gel in a minigel apparatus. Restriction fragments were detected by staining with ethidium bromide.

Results

1. Determination of HLA-DPB1 Gene Types

HLA-DPB1 gene types were determined by comparing them to the RFLP's patterns of amplified DPB1 gene reported by Ota et al.

2. Distribution of the HLA-DPB1 Gene Locus

The percentage of HLA-DPB*0201,*0202,*0301,*0401,*0402,*0501,*0601,*0901,*1001,*1401,*1601,*1901 in the APA-S group were 43.6%, 5.1%, 15.4%, 2.6%, 17.9%, 61.5%, 2.6%, 7.7%, 2.6%, 12.8%, 2.6%, respectively. When compared to the control group, there was no significant difference between them.

Discussion

Recently, it has been reported that abnormalities of autoimmune mechanisms have been associated with recurrent abortion. These abortions are highly associated with the existence of antiphospholipid antibodies (APA). It has been suggested that APA promotes thrombosis and these clinical symptoms are induced. In this point of view, APA-S is considered as one of the autoimmune diseases. There are few reports about the association of HLA-DP antigen and APA-S. For a long time the HLA-DP antigen has been defined by the primed lymphocyte typing (PLT) technique using DP-specific PLT cells. However, PLT-based DP typing is not technically simple. Recently the PCR-RFLP method provides a simple and accurate procedure for determination of the HLA class II types at the nucleotide level. We adopted this method for the HLA-DPB1 genes. As the result, there was no significant difference between the APA-S group and the control group for the HLA-DPB1 genes. For the further analysis about the association of HLA and APA-S, it is necessary to determine the other class II type such as HLA-DR gene.

References