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The role of HLA-DRB1 alleles on susceptibility of Chinese patients with anti-GBM disease

Rui Yang, Zhao Cui, Juan Zhao, Ming-Hui Zhao *

Renal Division, Department of Medicine, Peking University First Hospital, Institute of Nephrology, Peking University, Key Laboratory of Renal Disease, Ministry of Health of China, Beijing 100034, China

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Abstract Anti-glomerular basement membrane (GBM) disease, a rare autoimmune disorder, is associated with HLA-DR15 genotype in Caucasian and Japanese populations. But the distribution of HLA-DRB1 alleles in Chinese patients with anti-GBM disease and their association with clinical characteristics of anti-GBM disease are to be determined. The present study analyzed the HLA-DRB1 alleles by sequence based typing in 44 Chinese patients with anti-GBM disease and 200 healthy controls. The effects of DRB1 alleles on susceptibility to anti-GBM disease were examined by a relative predispositional effects (RPEs) method. The clinical and pathological data of the patients were collected and analyzed. The DRB1*1501 allele was significantly associated with anti-GBM disease ($p = 1.597 \times 10^{-7}$). The RPEs test also showed a significant increased frequency of DRB1*0404 in anti-GBM disease ($p = 0.037$). Interestingly, the patients with DRB1*1501 or *0404 had more crescent formation in glomeruli than those without the two alleles ($p = 0.021$). But the DRB1*0404 was rare in both patients and control groups, which indicates that the importance of the *0404 allele is limited in anti-GBM disease. We conclude that the HLA-DRB1*1501 allele is a genetic marker for susceptibility to anti-GBM disease.

Introduction

Anti-glomerular basement membrane (GBM) disease is a rare autoimmune disorder which is characterized by the production of autoantibodies directed to the GBM, rapidly progressive glomerulonephritis and a high risk for alveolar hemorrhage. Anti-GBM autoantibodies play a central role in the pathogenesis of anti-GBM disease [1,2]. The target autoantigen has been identified as the α3 chain non-collagen 1 domain of type IV collagen [α3(IV)NC1] [3]. Two conformational epitopes of anti-GBM autoantibodies had been defined as E_A and E_B [4,5].

Although the etiology remains unknown, it is widely believed that the immune tolerance was broken in anti-GBM patients after exposure to as-yet undefined environmental factors [6]. Once the immune tolerance was broken, α3(IV)NC1 was recognized as a neoantigen by the immune system of the patients. The T-cell epitopes, linear peptides derived from α3(IV)NC1, will bind to the major histocompatibility complex (MHC) molecules and are presented to T cells, which is critical to the expansion and differentiation of B cells into antibody producing plasma cells. The definition of the T-cell epitopes makes it attractive to study anti-GBM disease in association with MHC.

The MHC genes, also called human leukocyte antigen (HLA) genes in human, located on the short arm of chromosome 6, encode numerous molecules that have immuno-

* Corresponding author. Fax: +86 10 66551055.
E-mail address: mhzhao@bjmu.edu.cn (M.-H. Zhao).

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logical functions including the HLA class I and II molecules. The HLA genes have been associated with most, if not all, autoimmune diseases [7]. HLA genetic susceptibility to anti-GBM disease has been studied in different populations, including British, American, French, Australian and Japanese [8–12]. In 1978, the association between HLA-DR2 and anti-GBM disease was initial defined by a serological study [13]. Later, DR2 was split into DR15 and DR16, and DR15 was demonstrated to be the most frequent genotype [8]. As the development of DNA sequence technique, HLA-DRB1*1501 was identified to be the major risk allele [9,10,12]. But there is no data of the HLA genotypes in Chinese patients with anti-GBM disease. The present study was to examine the distribution and clinical association of HLA-DRB1 alleles in Chinese patients with anti-GBM disease.

Methods

Patients and controls

This study included 44 hospitalized patients with anti-GBM disease, who were diagnosed at our referential diagnostic center in Peking University First Hospital from 1996 to 2007. The diagnosis was established in all the cases by the presence of glomerularnephritis and the detection of serum anti-GBM autoantibodies by ELISA. All the 44 patients were performed renal biopsy.

The control group comprised 200 healthy individuals, who were ethnically matched, voluntarily recruited and selected among Chinese blood donors.

Samples

Peripheral blood samples (10 ml) from patients with anti-GBM disease and normal controls were collected in EDTA. Genomic DNA was obtained from peripheral blood leukocytes with a salting-out procedure [14].

Sequence based typing

Typing of HLA-DRB1 alleles was performed by bi-directional sequencing of exon 2 using the SeCoreTM Sequencing Kits (Invitrogen, Brown Deer, WI, USA).

Clinical and pathological data

Data upon diagnosis and during follow-up were collected from Peking University First Hospital. Clinical data, including serum creatinine, presence of lung hemorrhage and urinary output were collected upon diagnosis. Renal histopathologic data included light microscopy and direct immunofluorescence microscopy. Follow-up data were collected including endpoint events: dialysis dependency or death at the end of one year after diagnosis.

Detection of serum anti-GBM autoantibodies and anti-neutrophil cytoplasmic autoantibodies

Anti-GBM autoantibodies were measured by ELISA using bovine α(IV)NC1 as the solid phase antigen, which was described previously [15]. The results were expressed as relative absorbance value to a reference positive serum and values greater than 0.13 were regarded as positive.

Anti-neutrophil cytoplasmic autoantibodies (ANCA) were screened by indirect immunofluorescence assay (IIF), and antigen specificities were determined using ELISA for anti-MPO and anti-PR3 antibodies as previously described [16].

Statistical analysis

The difference in the frequencies of HLA antigens in disease samples and controls was compared using the chi-square test or Fisher's exact test as appropriate. We used a relative predispositional effects (RPEs) method to examine the relative effects of individual DRB1 alleles on susceptibility to anti-GBM disease [17]. To compare the DRB1 status of subjects stratified by gender, age and indices of severity of anti-GBM disease, chi-square test, Fisher's exact test, nonparametric Mann-Whitney test or Student's t test were used as appropriate.

Results

Demographic and clinicopathological features

Forty-four patients with anti-GBM disease, diagnosed in Peking University First Hospital from 1996 to 2007, were included in the present study. Among the 44 patients with anti-GBM disease, 30 were males and 14 were females. The median age of the 44 patients was 27 (ranged from 13 to 82) years old on diagnosis. All of the 44 patients had hematuria and proteinuria. Lung hemorrhage was evident in 36.3% (16/44) of patient. Seventeen out of the 44 patients had anuria/oliguria. The mean serum creatinine on diagnosis was 765.4 ± 388.7 μmol/L.

Renal biopsy was performed in all the 44 patients. Forty-one (93.8%) patients had crescent formation in more than 50% of the glomeruli and 30 (68.2%) had crescent formation in more than 85% of the glomeruli. Two had crescents in less than 50% of the glomeruli and the rest one had mild mesangial lesion only. Direct immunofluorescence examination was performed in 35 cases. All of them showed linear or fine granular IgG and/or C3 deposition along glomerular capillary wall.

Outcome data were available for 40 out of the 44 patients. At the end of 1 year after diagnosis, only 7/40 (17.5%) patients were dialysis independent, and 33/40 (82.5%) patients were dialysis dependent or died.

Anti-GBM autoantibodies and ANCA

Levels of anti-GBM autoantibodies in sera were measured by ELISA and were expressed as relative absorbance value (Fig. 1). Ten of the 40 patients were also ANCA positive. Among the 10 ANCA positive patients, nine recognized myeloperoxidase and one recognized proteinase 3.

HLA-DRB1 alleles and susceptibility to anti-GBM disease

The phenotype frequencies of each HLA-DRB1 allele for the 44 anti-GBM patients and 200 ethnically matched healthy