Is Tumor Necrosis Factor Genotype (TNFA2/TNFA2) a Genetic Prognostic Factor of an Unfavorable Outcome in IgA Nephropathy?

The purpose of this study was to examine whether there are the associations between TNF α and TNF β gene polymorphisms and the development and progression of IgA nephropathy (IgAN). A cross-sectional study on TNF α and TNF β gene polymorphisms by polymerase chain reaction with restriction fragment length polymorphisms was performed on 76 patients with primary IgAN confirmed by renal biopsy and 100 healthy controls. The allele with G→A substitution was designated as TNFA2 for the TNF α gene and TNFB2 for the TNF β gene. A patient in whom dialysis treatment was started or whose serum creatinine became double or over during the follow-up duration was designated as a “progressor”. The TNFA2/TNFA2 genotype was more prevalent in the progressor than in the non-progressor group (20.0 vs 3.3%, p<0.05). Clinical factors such as serum creatinine, systolic and diastolic blood pressure (p<0.001, respectively) were higher and pathologic factor such as Grade IV or V renal lesions was more prevalent (p<0.01) in the progressor than in the non-progressor group. Therefore, TNFA2/TNFA2 genotype may be a risk factor for the progression of IgAN.

Key Words : Glomerulonephritis, IgA; Tumor Necrosis Factor; Polymorphism (Genetics)

INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is the most common form of primary glomerulonephritis and is an important cause of end-stage renal disease requiring renal replacement therapy (1). Although the pathogenetic mechanism of IgAN is still controversial, a great body of evidence suggests that cytokines such as tumor necrosis factor (TNF) and interleukin play an important role in the renal injury of IgAN (2, 3).

TNFα and TNFβ are potent cytokines which are associated with the inflammatory response (4). TNF is involved in the initiation or progression of renal injury (5). Increased expression of TNF has been demonstrated in vivo in several animal models of glomerulonephritis (6). TNFα and TNFβ genes are closely linked and tandemly arranged in the human genome and map between class I and class III regions of major histocompatibility complex (MHC) in humans (4). The location of TNF genes within the MHC raised a possibility that some MHC-linked inflammatory diseases might be related to genetic variations inside the TNF locus. A genomic polymorphism within the TNF locus influences plasma TNF concentration (7). It is known that there are at least two polymorphisms in the class III region controlling TNFα production. One is at position -308 base pairs in the promoter region of the TNFα gene (7), for which allele 2 (TNF2) is associated with higher constitutive and inducible levels of TNFα (8). The other is in intron 2/exon 3 of the TNF gene, with the two polymorphic alleles variably associated with high and low levels of TNFα secretion by mononuclear cells, depending on the population under investigation (9, 10). However, the role of TNF gene polymorphism in IgAN is not clear as yet.

We evaluated the G→A transition polymorphism of the TNFα and TNFβ gene with NcoI restriction fragment length analysis and their clinical significances in seventy-six patients with primary IgAN confirmed by renal biopsy.

MATERIALS AND METHODS

A total of 76 patients (38 males, 38 females; mean age, 30.4 yr) diagnosed as IgA nephropathy by renal biopsy were included in this study. We excluded patients with glomerulonephritis associated with diabetes mellitus, systemic lupus erythematosus, or hepatitis B or C virus. One hundred healthy volunteers (49 males, 51 females; mean age, 48.2 yr) were recruited during an annual physical examination at Soonchunhyang Chonan Hospital (Chonan, Korea) as normal controls.

Genomic DNA was extracted by DNA extraction column (QI Amp blood kit; Qiagen, Venlo, The Netherlands) from
Peripheral blood. Polymerase chain reaction (PCR) was performed using the primer sets under the conditions previously described (7, 11): sense primers (TNFα: 5'-AGG CAA TAG GTT TTG AGG GCC AT-3'; TNFβ: 5'-CGG TGC TTC GTG CTT TGG ACT A-3'), antisense primers (TNFα: 5'-TCC TCC CTG CTC CGA TTC CG-3'; TNFβ: 5'-GAG GGG GTG GAT GCT TGG GCT C-3'), and a GeneAmp PCR machine (Perkin Elmer 9600; Perkin Elmer, Norwalk, CT) were used. Products of 107 and 782 bp were generated for TNFA and TNFB, respectively. The amplified PCR products were digested with Nol and analysed by gel electrophoresis. For TNFα, primers were designated to incorporate a polymorphic site at a position -308 bp of the TNFα gene into a Nol restriction site. Restriction digests generated products of 87 and 20 bp for allele 1 (TNFA1) and 107 bp for allele 2 (TNFA2). The 782 bp fragment amplified across intron 2 of the TNFβ gene also incorporates a polymorphic Nol site, which generates fragments of 782 bp for TNFB2 and 586 plus 196 bp for TNFB1. Ethidium bromide staining of the gel demonstrated the original 782 bp fragment (homozygous patients for allele TNFB2, lacking the Nol site), three fragments of 782, 586, and 196 bp of length (heterozygous patients), or two fragments of 586 and 196 bp of size (homozygous patients for the allele TNFB1) (Fig. 1). The clinical parameters at renal biopsy and the latest follow-up by IgA nephropathy at renal biopsy and the lastest follow-up by TNFα genotypes.

\[ \text{p-value was calculated by the Student's t-test for continuous variables or Fisher's exact test for categorical variables. } p<0.05 \text{ was considered to be statistically significant.} \]

Table 1. Distribution of tumor necrosis factor (TNF) \(\alpha\) and TNF \(\beta\) gene frequencies in control subjects and patients with IgA nephropathy

|       | Control | IgA nephropathy |
|-------|---------|-----------------|
|       | TNFα    |                 |                 |
| Genotype | TNFα1/TNFα1 | 65 (65.0) | 45 (59.2) |
|         | TNFα1/TNFα2 | 31 (31.0) | 26 (34.2) |
| Allele  | TNFα1   | 161 (80.5) | 116 (76.3) |
|         | TNFα2   | 39 (19.5)  | 36 (23.7)  |
| Genotype | TNFβ1/TNFβ1 | 4 (4.0)   | 8 (10.5)   |
|         | TNFβ1/TNFβ2 | 57 (57.0) | 37 (48.7)  |
| Allele  | TNFβ1   | 65 (32.5)  | 53 (34.9)  |
|         | TNFβ2   | 135 (67.5) | 99 (65.1)  |

Values are expressed as number (percent). There were no significant statistical differences between the groups.

RESULTS

Polymorphism of the TNFα gene

The genotype of TNFα distribution was as follows: in the 76 patients with IgAN, 45 patients (59.2%) had the TNFα1/TNFα1 genotype, 26 patients (34.2%) had the TNFα1/TNFα2 genotype, and 5 patients (6.6%) had the TNFα2/TNFα2 genotype. There was no significant difference in the distribution of genotype or allele frequencies of the TNFα gene between IgAN patients and control subjects (Table 1). There was no significant difference in clinical parameters which included the age, 24-hr proteinuria, serum creatinine, hematocrit, cholesterol, and systolic and diastolic BP at renal biopsy among the three TNFα genotypes. And neither was there any difference in the follow-up duration of the three

![Fig. 1. Electrophoresis of the PCR product of the TNF α and TNF β genes. Lane 1 is a homozygote of TNFα2, lane 2 is a homozygote of TNFα1, and lane 3 is a heterozygote of TNFα1 and TNFα2. Lane 4 is a homozygote of TNFβ2, lane 5 is a homozygote of TNFβ1, and lane 6 is a heterozygote of TNFα1 and TNFα2.](image-url)

Table 2. Comparison of clinical characteristics in patients with IgA nephropathy at renal biopsy and the lastest follow-up by TNFα genotypes

|       | TNFα1/TNFα1 | TNFα1/TNFα2 | TNFα2/TNFα2 |
|-------|-------------|-------------|-------------|
| (n=45) | (n=26)      | (n=5)       |
|       | At renal biopsy |             |             |
| Age (yr) | 31.4 ± 10.2 | 31.9 ± 10.2 | 36.8 ± 10.7 |
| Proteinuria (mg/24 hr) | 2,428 ± 2,737 | 3,164 ± 3,519 | 5,240 ± 3,150 |
| Serum creatinine (mg/dL) | 1.2 ± 0.5 | 1.1 ± 0.4 | 1.2 ± 0.6 |
| Systolic BP (mmHg) | 131.2 ± 22.6 | 138.1 ± 18.8 | 144 ± 18.1 |
| Diastolic BP (mmHg) | 83.8 ± 15.3 | 85.8 ± 12.1 | 94 ± 15.2 |
| Hematocrit (%) | 40.0 ± 5.3 | 40.0 ± 4.2 | 43.8 ± 7.5 |
| Cholesterol (mg/dL) | 216.7 ± 54.9 | 202.1 ± 49.1 | 199.2 ± 52.2 |
| At follow-up |             |             |             |
| Progressor (%) | 22.2 | 7.7* | 60.0* |

Values are expressed as mean ± S.D. or percent. p-value was calculated by ANOVA for continuous variables or Fisher’s exact test for categorical variables. *p<0.05. A patient in whom dialysis treatment was started or whose serum creatinine became double or over during the follow-up duration was designated as “progressor”.

|       |       |       |
|-------|-------|-------|
|       |       |       |
|       |       |       |
follow-up duration was designated as 37.3 months. The systolic and diastolic blood pressures at renal biopsy were significant higher in the progressor group than non-progressor group (p<0.001, Table 4) and at the latest follow-up (1.28±1.13 vs 6.09±2.11, p<0.001) were higher in the progressor group than in the non-progressor group. The rate of decline of renal function was calculated as [(1/serum creatinine at renal biopsy-1/serum creatinine at the latest follow-up)/months of follow-up duration] × 100. “Grading system according to Lee (12) was available only for 49 patients due to insufficient kidney biopsy material. p-value was calculated by Student’s t-test for continuous variables or Fisher’s exact test for categorical variables, *p<0.05, †p<0.01, ‡p<0.001.

Polymorphism of the TNFβ gene

The genotype of TNFβ distribution were as follows: in the patients with IgAN, 8 patients (10.5%) had the TNFB1/TNFβ1 genotype, 37 patients (48.7%) had the TNFB1/TNFβ2 genotype, and 31 patients (40.8%) had the TNFB2/TNFβ2 genotype. There was no significant difference in the distribution of the genotype of the TNFβ gene between the IgAN and control subjects (Table 1). The overall allele frequency of TNFβ was also shown in Table 1. There were no significant differences in the clinical parameters which included the age, 24-hr proteinuria, serum creatinine, hematocrit, cholesterol, systolic and diastolic BP at renal biopsy (Table 3), follow-up duration and the frequency of those belonging to the progressor group among the three TNFβ genotypes (52.0±27.4 vs 57.8±37.9 vs 64.6±41.2 months).

Prognostic factors of progression of IgAN

The follow-up duration was not significantly different between the non-progressor and progressor groups (54.8±36.9 vs 80.0±37.3 months). The systolic and diastolic blood pressure at renal biopsy were significant higher in the progressor group than non-progressor group (p<0.001, respectively, Table 4). The proteinuria at renal biopsy was higher in the progressor group than non-progressor group, however, it did not reach the statistical significance (Table 4). There were no significant differences in the age, hematocrit, and cholesterol at renal biopsy between the groups (Table 4). The proteinuria at renal biopsy was higher in the progressor group than non-progressor group (p<0.001, Table 4). In the progressor group, 12 patients with end-stage renal disease were included the age, 24-hr proteinuria, serum creatinine, hematocrit, cholesterol, systolic and diastolic BP at renal biopsy included the age, 24-hr proteinuria, serum creatinine, hematocrit, cholesterol, systolic and diastolic BP at renal biopsy (Table 4). The proteinuria at renal biopsy was higher in the progressor group than non-progressor group, however, it did not reach the statistical significance (Table 4). There were no significant differences in the age, hematocrit, and cholesterol at renal biopsy between the groups (Table 4). The proteinuria at renal biopsy was higher in the progressor group than non-progressor group (p<0.001, Table 4).

The genotype of TNFβ gene between the non-progressor and progressor groups. The rate of decline of renal function was calculated as [(1/serum creatinine at renal biopsy-1/serum creatinine at the latest follow-up)/months of follow-up duration] × 100. “Grading system according to Lee (12) was available only for 49 patients due to insufficient kidney biopsy material. p-value was calculated by Student’s t-test for continuous variables or Fisher’s exact test for categorical variables, *p<0.05, †p<0.01, ‡p<0.001.

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cluded. Nine patients had the TNFA1/TNFA1 genotype, 2 patients had the TNFA1/TNFA2 genotype, and 1 patient had the TNFA2/TNFA2 genotype. The TNFA2/TNFA2 genotype was more prevalent in the progressor group than non-progressor group (p<0.05, Table 4, Fig. 2).

**DISCUSSION**

TNFα is a proinflammatory cytokine that has been involved in certain forms of immune-mediated renal injury, including IgAN (5). TNFα is synthesized by glomerular cells or circulating macrophage/monocytes (13, 14). TNF activates mesangial cells to release procoagulants, plasminogen activator, and plasminogen activator inhibitor (13). Increased expression of TNF has been demonstrated in vivo in several animal models of glomerulonephritis including murine lupus models (6). The administration of TNF potentiates renal injury in IgAN, murine lupus models, and anti-GBM antibody disease. The location of TNF genes within the MHC raised a possibility that some MHC-linked inflammatory diseases might be related to genetic variations inside the TNF locus. The polymorphism of gene encoding TNFα at position -308 in the promoter region involving a transition of guanidine to adenosine has been characterized (11). The -308A polymorphism (TNFA2) is associated with an increase of TNFα transcription (15), which may predispose humans to the occurrence of inflammatory diseases. Carriage of the TNFA2 allele has been shown to be associated with several inflammatory diseases (16). There was no significant difference in the distribution of genotype or allele frequencies of the TNFα gene between IgAN patients and control subjects in this study. Our result was similar to previous reports (17). Therefore, we speculate that the TNFα gene polymorphism is not associated with the development of IgAN.

The clinical course of IgAN is extremely variable. The clinical predictors including impairment of renal function, severe proteinuria, and arterial hypertension at presentation have been known as an unfavorable outcome of IgAN (18). Our data supported these previous observations. The clinical parameters including serum creatinine, systolic and diastolic blood pressure at renal biopsy and the prevalence of Grade IV or V renal pathologic lesions were significantly higher in the progressor group than in the non-progressor group in this study. These also support previous results (18).

Besides clinical and pathologic prognostic factors, the interest to search for genetic markers such as HLA-B35 or specific genotype of angiotensin converting enzyme (ACE) polymorphism for progression of IgAN has increased. However, no study has confirmed the association between the progression of IgAN and the presence of specific HLA or ACE genotypes (18). Also, the prognostic significance of TNFα gene polymorphism in IgAN patients on the progression of the disease has not been described so far. The patients with IgAN who had a progressive disease were more commonly observed in the subgroup of the TNFA2/TNFA2 genotype and the TNFA2/TNFA2 genotype was more prevalent in the progressor group than in the non-progressor group in our study. This is the first report, to our knowledge, that the TNFA2/TNFA2 genotype is a genetic prognostic factor in IgAN.

There are some limitations in this study. Firstly, the number of patients with TNFA2/TNFA2 was too small to perform multiple logistic regression analysis. Secondly, we used the changes of 1/(serum creatinine) as the parameter of renal progression of IgAN because other parameters such as creatinine clearance were not available. Thirdly, this was a cross-sectional study, and we obtained the data retrospectively. Therefore, there might have been some bias for the inclusion of patients. Fourthly, all study subjects are Koreans in our study, therefore, we could not rule out possible ethnic differences. Lastly, we did not analyze the influence of various treatments.

The TNFβ gene is closely linked to TNFα, tandemly arranged in the human genome, and map between class I and class III regions of MHC in humans (4). The two Nod-defined TNFB alleles, which differ in structure and in the level of TNFβ inducibility, might be relevant for MHC-associated predispositions for autoimmune diseases (8). The TNFB1 allele is strongly linked to the altered production of TNFβ and the variant polypeptide structure might contribute to disease susceptibility of this haplotype. It is conceivable that an allelically varying TNFβ response of activated T lymphocytes might contribute to the inflammatory mechanism of local autoimmune reactions (8). Previous reports demonstrated an association between a Nod TNFβ polymorphism and immune-mediated glomerulonephritis including membranous nephropathy and IgAN. They explained this association by linkage disequilibrium with extended MHC haplotypes, however, whether the TNFβ polymorphism per se is involved in disease susceptibility is not clear in IgAN (10). We could not find any evidence for the association between TNFβ polymorphism and IgAN in this study.

In conclusion, this is the first report showing the association between the specific TNF genotype and the progression of IgAN. The polymorphisms in the TNFα and TNFβ gene were not associated with the development of IgAN, however, the TNFA2/TNFA2 genotype of the TNFα gene was more commonly found in patients with IgAN who had a progressive disease than those with the TNFA1/TNFA1 or TNFA1/TNFA2 genotype. Therefore, although there were some limitations in this study, it is suggested that the TNFA2/TNFA2 genotype may be a risk factor for the progression of IgAN.

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