# Tumor Necrosis Factor-α Polymorphism and Mannose Binding Lectin Gene Mutations in Various Status of Hepatitis B Virus Infection

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# Tumor Necrosis Factor-α Polymorphism and Mannose Binding Lectin Gene Mutations in Various Status of Hepatitis B virus Infection

Directed by Professor Kwang-Hyub Han

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Hye-Young Chang

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This certifies that the Master's Thesis of Hye-Young Chang is approved.

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**The Graduate School**
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**December 2003**

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2003. 12. 17.

장혜영

# Table of contents

# List of figures

# List of tables

**Abstract**

# Tumor Necrosis Factor-α Polymorphism and Mannose Binding Lectin Gene Mutations in Various Status of Hepatitis B Virus Infection

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(Directed by Professor, Kwang-Hyub Han)

Molecular genetic studies have increased our understanding of the pathogenesis of viral hepatitis. Tumor necrosis factor (TNF)-α polymorphism in the promoter region at position -308 was reported to be associated with chronic hepatitis B infection. Mutation of mannose binding lectin (MBL) was also reported to be related with chronic hepatitis B probably due to immuodeficiency caused by low serum level of MBL. We conducted the present study to investigate the association of MBL gene mutations and TNF-α promoter polymorphism with host's susceptibility to various courses of HBV infection in Korean.

A total of 419 subjects were classified into three groups according to the various status of HBV infection; 1) unexposed group (UE) [n=89,

HBsAg(-), anti-HBc(-), anti-HBs(-)], 2) spontaneously cleared group (SC) [n=85, HBsAg(-), anti-HBc and anti-HBs(+)], 3) chronic carrier group (CC) [n=245, HBsAg(+), anti-HBc(+), anti-HBs(-)]. CC was further subdivided into two groups [① non-progressive group (NP, n=85); inactive carrier, ② progressive group (P, n=160); chronic hepatitis, cirrhosis or HCC]. TNF-α promoter polymorphisms and MBL gene mutations were analyzed by ABI Prism SNaPshot$^{TM}$ ddNTP primer extension kit. The frequency of G to A transition at -308 TNF-α gene was statistically higher in SC (25.9%), compared to CC (6.1%, $P<0.001$). The frequency of TNF-α -238 polymorphism was not different between the three groups. Substantial genetic association was also noted between TNF-α promoter -308 and HLA-DRB1*13. MBL gene codon 54 mutation was not associated with HBV clearance (P=0.132) and progression of HBV infection (P=0.136).

Our findings suggest the association between the TNF-α promoter polymorphism at position -308 and clearance of HBV infection. But MBL gene mutation at codon 54 showed no considerable differences among three groups, suggesting no correlation with viral clearance and disease progression.

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Key words: hepatitis B virus (HBV), tumor necrosis factor (TNF)-α, mannose binding lectin (MBL), genetic polymorphism

# Tumor Necrosis Factor-α Polymorphism and Mannose Binding Lectin Gene Mutations in Various Status of Hepatitis B Virus Infection

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## I. INTRODUCTION

An estimated 350 million people worldwide are carriers of hepatitis B virus (HBV) infection, and approximately 250,000 deaths occur each year as a consequence of fulminant hepatic failure, cirrhosis, and hepatocellular carcinoma (HCC).[1] The clinical course of HBV infection is extremely diverse from spontaneous recovery to chronic persistent infection that might progress to chronic hepatitis, cirrhosis, or HCC. The various course of HBV infection is caused by the interplay between HBV replication and the host immune response.[2-4]

Clearance of HBV necessitate a coordinated humoral and

cell-mediated immune response. Cytokines are soluble polypeptide molecules that mediate cell-to-cell communication and regulate the intensity and duration of the immune response. Cytokine response profiles from T helper cells are classified as Th1 responses that enhance cellular immunity [interferon (IFN)-$\gamma$, interleukin (IL)-2, tumor necrosis factor (TNF)-$\alpha$] or Th2 responses that enhance humoral immunity (IL-4, IL-5, IL-10). In acute self-limited HBV infection, most HBV DNA molecules are rapidly cleared in the incubation phase by a vigorous, multifaceted, polyclonal immune response to hepatitis B surface, core, and polymerase antigens.[5] Under these circumstances, a proinflammatory Th1 response with high serum and intrahepatic levels of IFN-$\gamma$ and TNF-$\alpha$ inhibits HBV gene expression and replication and leads to the rapid destruction and clearance of infected hepatocytes by both cytopathic and noncytolytic mechanisms.[6-8] Accordingly, various cytokines play an important role in the defense against HBV infection.

Chisari group first demonstrated the role of TNF-$\alpha$ on the clearance of HBV.[6-8] In an HBV transgenic mouse model, IFN-$\gamma$ and TNF-$\alpha$ secreted by cytotoxic T lymphocytes (CTLs) abolished HBV gene expression and replication without killing the hepatocyte, suggesting non-cytolytic clearance of HBV.[8] To date, there are several growing evidences that TNF-$\alpha$ plays an important role in the course of HBV infection.[9-12] However, little is known about the role of TNF-$\alpha$ promoter polymorphism as a host genetic factor in the course of HBV infection.

The gene encoding TNF-$\alpha$ is located within the class III region of the major histocompatibility locus (MHC) between HLA-B and HLA-DR (Fig. 1).[13]

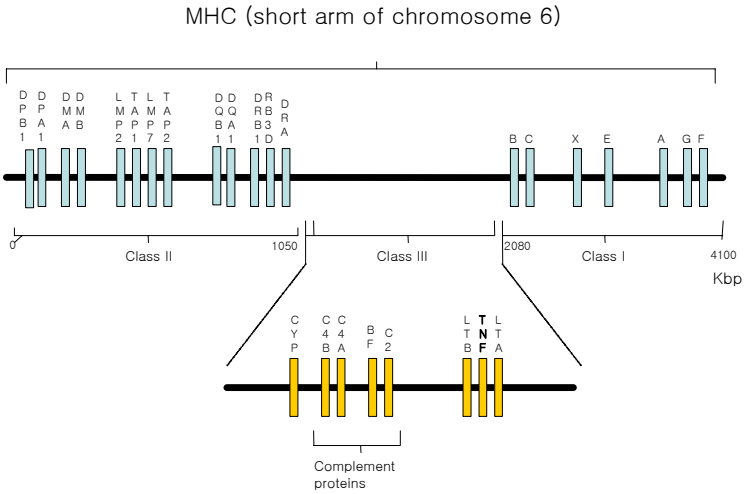


**Fig. 1. The genetic organization of the HLA and TNF genes. The gene encoding TNF-α is located within the class III region of MHC between HLA-B & HLA-DR.**

Recently there have been considerable interests in the relationship between TNF-$\alpha$ gene polymorphism and susceptibility/resistance to infectious diseases.[14,15] McGuire *et al*.[16] found an increased prevalence of homozygotes for a single base change at position -308 of the TNF promoter in children with cerebral malaria in the Gambia. This condition is associated with markedly elevated serum levels of TNF, and Wilson *et al.*[17] have reported that this promoter variant is a stronger transcriptional activator than the common allele. This -308 variant has also been associated with mucocutaneous leishmaniasis[18] and lepromatous leprosy[19], and high TNF levels seen in these diseases seem to be causally involved in pathogenesis rather than just a marker of an ongoing pathological process.

The G to A transitions in the promoter region at positions ▬163, ▬238, ▬308, and ▬376 are well-known polymorphisms. Two G *versus* A transitions in

the promoter region at positions -308 and -238 have shown to affect TNF-$\alpha$ expression *in vitro* and *in vivo* system.[11,17,18,20-22] Recently, Hőhler *et al*. showed that TNF-$\alpha$ promoter polymorphism at position -238 was closely linked to chronic HBV infection in a European.[11]

Mannose binding lectin (MBL), like the macrophage mannose receptor, binds carbohydrates with terminal mannose, which are typically found in microbial cell surface glycoprotein and glycolipids.[23] MBL is an important constituent of the human innate immune system and may play a role in HBV infection. MBL binds through multiple lectin domains to the carbohydrate moieties which are expressed on the surface of many microbial organisms and it is able to activate the complement system cascade.[24] The functional human MBL gene is located on chromosome 10 at position 10q11.2-q21 and consists of four exons.[25] Exon 1 encodes the signal peptide, the N-terminal cysteine-rich region and a collagen-like glycine-rich region. Single nucleotide polymorphisms (SNP) in the MBL gene found in exon 1 are the major determinants of MBL deficiency (Fig. 2).[26]

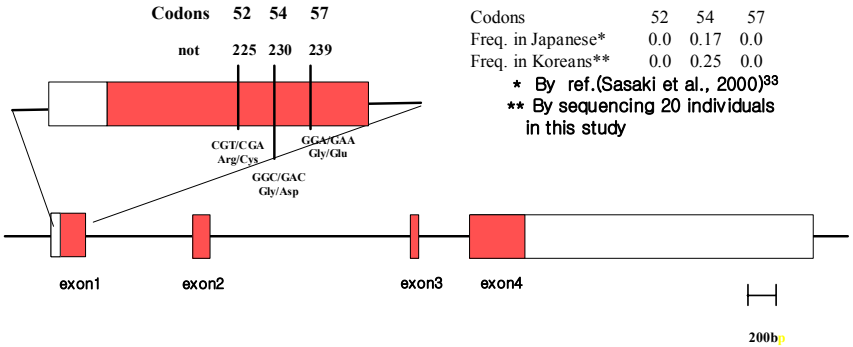


**Fig. 2. Map of human mannose binding lectin (MBL) on chromosome 10q11.2-q21. Coding exons are marked by shaded blocks and 5' and 3'UTR by white blocks. The size of full genome is about 7 kb.**

In 1996, Thomas *et al.*[27] reported an association of codon 52 mutation of the MBL gene with persistent HBV infection in Caucasian patients. And the association of MBL gene codon 54 mutation and progression of liver disease after HBV infection was reported by Hong Kong study.[28] This association may be due to an inability to fix complement, which is supported by the finding of a low serum opsonic index in patients with persistent HBV infection.

Korea is a highly endemic area of HBV infection. Five to 8% of Koreans are chronic HBsAg carriers. Since the Korean population is racially homogenous and most Korean carriers were HBV genotype C,[29] host genetic factors could be well investigated regardless of racial and virological differences. The aim of this study is to investigate whether TNF-α promoter polymorphisms and MBL

gene mutation in codon 54 influence the clinical courses of HBV infection.

# II. MATERIALS AND METHODS

## 1. Study subjects

A total of 419 Korean subjects having either present or past evidence of HBV infection were enrolled from the patients clinic of the liver unit or from Yonsei University Medical Center between March 2003 and August 2003. They were regularly followed up, with measurements of serum alanine aminotransferase (ALT) levels and HBV markers such as HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe using commercially available enzyme immunoassay kit (Abbott Diagnostics, North Chicago, IL, USA).

Subjects were classified into three groups as follows, "Unexposed group (UE)" contained 89 subjects, who were negative for all HBV markers suggesting no exposure to HBV before the test,"Spontaneously cleared group (SC)" contained 85 subjects, who were negative for HBsAg and positive for anti-HBs and anti-HBc, "Chronic carrier group (CC)" contained 245 patients, who had been HBsAg positive for at least 6 months. CC was further subdivided into two groups, "Non progressive group (NP)" consisted of 85 patients who were considered to be inactive HBsAg carrier with sustained normalization of the serum ALT level and negative for HBV DNA. 160 patients with chronic hepatitis B, liver cirrhosis and HCC belonged to "Progressive group (P)". Among CC group, the patients with HCC were also compared to the patients without HCC. MBL gene mutations were analyzed in a total of 273 patients.

All samples had been tested previously for HLA-DR. HLA-DR was

phenotyped serologically using PCR-single strand conformational polymorphism technique.

**2. The analysis of SNPs in TNF-α promoter gene and MBL genes**

Genomic DNA was extracted from whole blood using a Qiagen DNA Blood Mini Kit (Qiagen, Mildren, Germany) according to the manufacturer's instructions. A 328 bp fragment spanning position from -396 to -69 of the TNF-α gene was amplified using primers TNF-α-396 (5'-TTCCTGCATCCTGTC TGGAA-3') and TNF-α-69 (5'-CAGCGGAAAACTTCCTTGGT-3'). MBL genes were amplified by PCR using primer MBL-61 (5'-GAGGCCAGGGATG GGATGGGTCATC-3') and MBL-270 (5'-CCAACACGTACCTGGTTCCC -3').

PCR was performed with 20 pmol of each primer, 100 ng of genomic DNA, 250 μM dNTPs, and 0.5 unit of *Taq* DNA polymerase (Takara, OTSU, Japan) in the buffer provided by the manufacturer. Amplification was performed in a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, USA). The cycling conditions were used as follows; 94℃ for 10 min, followed by 35 cycles of 94℃ for 1 min, 55℃ for 1min and 72℃ for 1 min. A final extension step was 72℃ for 7 min.

Reactions were confirmed by gel electrophoresis with ethium bromide staining and PCR product was purified by a PCR purification kit (Qiagen, Mildren, Germany). To clean up the primer extension reaction, one unit of shrimp alkaline phosphatase (SAP; Amersham Life Sciencese, Clenveland ,OH,

USA) was added to reaction mixture and the mixture was incubated at 37℃ for 1 hour, followed by 15 min at 72℃ for enzyme inactivation. Primer extension reactions at both directions were performed with SNaPshot ddNTP Primer Extension Kit (Applied Biosystem, Foster City, CA, USA) according to the manufacture's instruction. To clean up the primer extension reaction, one unit of SAP was added to reaction mixture and the mixture was incubated at 37℃ for 1 hour, followed by 15 min at 72℃ for enzyme inactivation. The DNA sample, containing extension products and Genescan 120 Liz size standard solution were added to Hi-Di formamide (Applied Biosystems) by the recommendation of manufacturer. The mixture was incubated at 95℃ for 5 min, followed by 5 min on ice. The results were analyzed using the program of ABI Prism GeneScan and Genotyper (Applied Biosystems).

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**3. Statistical analysis**

All of statistical analysis was performed using Window SPSS release 10.0 for personal computer. The chi-square test and Fisher's exact method for small sample number were used.

$P < 0.05$ was regarded as significant.

# IV. RESULTS

## 1. The clinical characteristics of subjects

In a total of 419 Korean subjects, 293 were males and 126 were females. The mean age of total subjects was 42.5±10.6 years (range 18 - 63 years). UE, SC and CC consisted of 89, 85 and 245 subjects, respectively. Among 245 patients in CC, 85 and 160 patients belonged to NP and P respectively.

There was no significant difference of male-to-female ratio and age between SC and CC. However the patients in the progressive group (P) were older than the non-progressive group (NP) and male gender was important factor in the disease progression ($P<0.001$, Table 1).

**Table 1. The Characteristics of Subjects**

| | No. of Patients | | | Age (Mean ± SD) | |
|---|---|---|---|---|---|
| **Group** | **Total** | **Male** | **Female** | **Male** | **Female** |
| **UE** | **89** | **45** | **44** | **38.1±14.3** | **38.9±13.4** |
| **SC** | **85** | **60** | **25** | **42.9±14.3** | **47.7±14.9** |
| **CC** | **245** | **188** | **57** | **42.2±13.4** | **44.3±12.9** |
| **NP** | **85** | **54** | **31** | **38.9±12.9*** | **40.4±13.4** |
| **P** | **160** | **134** | **26** | **46.9±10.7** | **46.5±11.0** |
| **Total** | **419** | **293** | **126** | **41.7±10.5** | **43.3±12.3** |

**Note. UE (Unexposed group): HBsAg(-), anti-HBc(-), anti-HBs(-); SC (Spontaneously cleared group): HBsAg(-), anti-HBc and anti-HBs(+); CC (Chronic carrier group): HBsAg(+), anti-HBc(+), anti-HBs(-). CC was further subdivided into two groups; NP (Non progressive group): inactive carrier, P (Progressive group): chronic hepatitis, cirrhosis or HCC (hepatocellular carcinoma). Age of male gender showed a significant difference between NP and P (*P=0.001).**

## 2. The frequencies of TNF-$\alpha$ promoter polymorphism at positions -308 and -238

Twelve and 22 subjects (14.1 % and 25.9 %) in SC had TNF-$\alpha$ promoter polymorphism at positions -238 and -308, compared with 20 and 15 subjects in CC (8.2 % and 6.1 %). The frequency of TNF-$\alpha$ promoter polymorphism at position -308 in SC was significantly higher than that of CC (25.9 % vs 6.1 %, $P < 0.001$).

However, we found no significant differences in the frequency of TNF-$\alpha$ promoter polymorphism at -238 between analyzed groups.

In CC, there was no difference between NP and P in the frequency of the polymorphisms at position -238 (10.6 % vs 6.9 %, P = 0.220) and -308 (3.5 % vs 7.5 %, P = 0.171, Table 2).

**Table 2. The frequencies of TNF-$\alpha$ promoter gene polymorphism in UE, SC and CC**

| Genotype | UE n=89 (%) | SC n=85 (%) | CC n=245 (%) | | |
|---|---|---|---|---|---|
| | | | NP n=85 | P n=160 | Total n=245 |
| **TNF -238** | | | | | |
| G/G | 85 (95.5) | 73 (85.9) | 76 (89.4) | 149 (93.1) | 225 (91.8) |
| G/A or A/A | 4 (4.5) | 12 (14.1) | 9 (10.6) | 11 (6.9) | 20 (8.2) |
| **TNF -308** | | | | | |
| G/G | 77 (86.5) | 63 (74.1) | 82 (96.5) | 148 (92.5) | 230 (93.9) |
| G/A or A/A | 12 (13.5)* | 22 (25.9)† | 3 (3.5) | 12 (7.5) | 15 (6.1) |

**Note. UE (Unexposed group), SC (Spontaneously cleared group), CC (Chronic carrier group), NP (Non progressive group), P (Progressive group).**

**The frequency of G to A transition at -308 was statistically higher in SC, compared to CC († P<0.001), also was a significant difference between UE and CC (* P<0.05).**

**3. The frequenices of TNF-$\alpha$ promoter polymorphism at position -238 and -308 between patients with or without hepatocellular carcinoma (HCC)**

In 71 patients with HCC, 3 (4.2 %) patients had TNF-$\alpha$ promoter polymorphism, compared to 17 (9.8 %) patients without HCC at position -238 (P=0.150). No difference was also seen with -308 of TNF-$\alpha$ gene polymorphism (P = 0.701, Table 3).

**Table 3. The comparison of TNF-$\alpha$ promoter gene polymorphism between patients with HCC and without HCC in CC**

| | CC n=245 (%) | | P-value |
|---|---|---|---|
| | with HCC n=71 | without HCC n=174 | |
| **TNF -238** | | | |
| **G/G** | **68 (95.8)** | **157 (90.2)** | |
| **G/A or A/A** | **3 (4.2)** | **17 (9.8)** | **0.150** |
| **TNF -308** | | | |
| **G/G** | **66 (93.0)** | **164 (94.3)** | |
| **G/A or A/A** | **5 (7.0)** | **10 (5.7)** | **0.701** |

**Note: CC (Chronic carrier group), HCC (hepatocellular carcinoma)**

### 4. Linkage disequilibrium between TNF-$\alpha$ promoter polymorphisms and HLA-DRB1*13 in HBV infection

A statistically significant relationship was seen between the -308 polymorphism in the promoter region of TNF-$\alpha$ gene and HLA-DRB1*13 suggesting a strong linkage disequilibrium (Table 4).

**Table 4. The correlation between TNF-$\alpha$ promoter polymorphism and HLA-DR B1*13**

| HLA-DR B1*13 | TNF-$\alpha$ -238 | | | TNF-$\alpha$ -308 | | |
|---|---|---|---|---|---|---|
| | G/G | G/A or A/A | P | G/G | G/A or A/A | P |
| Negative | 335 (87.1) | 32 (89.7) | | 340 (91.9) | 27 (55.1) | |
| Positive | 48 (12.9) | 4 (10.3) | 0.340 | 30 (8.1) | 22 (44.9) | 0.001 |

**Note. Number in parentheses represent percentage values.**

**5. The frequencies of MBL gene mutation in various status of HBV infection**

Codon 57 and codon 52 mutations were absent in our preliminary results (Fig. 2). These mutation seem to be rare or absent in the Korean population. However, codon 54 mutation was common in this population .

The frequency of the codon 54 mutation was similar between the HBV infected group and UE. A allele (G/A or A/A) found in 29.7% of the UE and in 35.8% of SC, with no significant difference. We then investigated the relationship between MBL gene mutation and progression of liver disease. But, MBL gene mutations did not associated with the susceptibility of persistence or progression [NP(32.1%) vs. P(29.2%), P=0.084] in HBV infection (Table 5).

**Table 5. MBL gene mutation in various status of HBV infection**

| MBL gene codon 54 | UE n=37 (%) | SC n=53 (%) | CC n=183 (%) | | |
|---|---|---|---|---|---|
| | | | NP n=53 | P n=130 | Total n=183 |
| G/G | 26 (70.3) | 34 (64.2) | 36 (67.9) | 92 (70.8) | 128 (69.9) |
| G/A or A/A | 11 (29.7) | 19 (35.8)* | 17 (32.1)† | 38 (29.2) | 55 (30.1) |

**Note. UE (Unexposed group), SC (Spontaneously cleared group), CC (Chronic carrier group), NP (Non progressive group), P (Progressive group).**

**There were no significant difference between intergroups (*SC vs CC;P=0.132, † NP vs P; P=0.084).**

**6. The frequence of MBL gene mutation between patients with or without hepatocellular carcinoma (HCC)**

Fifteen of the 62 patients (24.2%) with HCC had codon 54 mutation. There was significant difference when it compared with the mutation rate of the patients without HCC (33.1%). Codon 54 mutation of MBL gene might be related to the development of HCC (P=0.015, Table 6).

**Table 6. The comparison of MBL gene mutation between patients with and without HCC in CC**

| MBL gene codon 54 | CC n=183 (%) | | P value |
|---|---|---|---|
| | with HCC n=62 | without HCC n=121 | |
| G/G | 47 (75.8) | 81 (66.9) | |
| G/A or A/A | 15 (24.2) | 40 (33.1) | 0.015 |

**Note: CC (Chronic carrier group), HCC (hepatocellular carcinoma)**

# IV. DISCUSSION

The number of studies on immunogenetic factors in infectious diseases has recently increased as more polymorphisms are identified in genes considered to have important roles in the pathogenesis of infectious diseases. Among these MHC, cytokine, and chemokine genes, their receptors have been the major targets of such studies.[43,44]

In an earlier study, Ahn *et al.*[30] reported that HLA-DR 13 is an important host factor influencing the immune response to HBV infection, suggesting the association with the elimination of HBV. Our efforts to unveil another immunogenetic factors responsible for the disease progression of HBV led to this study, demonstrating that TNF-$\alpha$ gene promoter -308 polymorphism was associated with the clearance of HBV, but not with the progression to liver cirrhosis or HCC.

The importance of IFN-$\gamma$ and TNF-$\alpha$ for the clearance of HBV has recently been emphasized. Penna *et al.*[31] reported that the antigen-specific fraction of peripheral blood T cells in acute self-limited HBV infection selectively secrete Th1-type cytokines, with a predominance of TNF-$\alpha$. This finding suggested that Th1-mediated effects may contribute not only to liver cell injury, but also to recovery and successful control of infection. In addition, these individuals show a strong CTL response directed against multiple epitopes[32,34] that are present in the core, envelope, and polymerase genes. These HBV-specific CTLs play a critical

role in clearance of the virus. By contrast, lymphoid cell clones from persons with chronic HBV produce a predominantly type 2 response.[35] Additionally, it has been shown that TNF-$\alpha$ can inhibit the replication of HBV-infected cells,[36] directly reducing viral load.

*In vitro* recombinant TNF-$\alpha$ inhibits HBV replication[47] through a post-translational mechanism that accelerates the degradation of HBV messenger RNA.[37] Furthermore, the core promoter element is sensitive to TNF-$\alpha$.[36] Ohta *et al.*[38] reported that both IFN-$\gamma$ and TNF-$\alpha$, which are produced by HBsAg-specific Th1 cells, seem to be indispensable during the effector phase of fulminant hepatitis. This was supported by studies in transgenic mice showing that intrahepatic induction of IFN-$\gamma$ and TNF-$\alpha$ downregulated HBV replication noncytopathically.[39-41] They mediated most of the antiviral effect of the CTLs, and hepatic induction of TNF-$\alpha$ was especially sufficient to inhibit HBV replication.[41]

Hőhler *et al*. reported the relationship between TNF-$\alpha$ promoter polymorphism at position -238 and the progression of chronic HBV infection,[11] and hepatitis C infection.[12] Their study was on Caucasian groups with diverse ethnic back ground and they did not investigate the relationship between the various diseases course of chronic HBV infection and TNF-$\alpha$ gene polymorphisms unlike this study. In our study, chronic carrier group was stratified into two groups, non-progressive and progressive group, and the relationship with HCC was also investigated.

We identified that the TNF-$\alpha$ gene polymorphisms involving G to A allele

transition at position -308 were more significantly found in SC than CC, suggesting the role of TNF-$\alpha$ for the clearance of HBV. Activation of CTLs, which is mediated by TNF-$\alpha$, was reported to play an important role in the resolution of HBV infection.[42] Although we did not check TNF-$\alpha$ level according to TNF-$\alpha$ polymorphism, the previous *in vitro* study[17] reported that the polymorphism at -308 had a significant effect on transcriptional activity in reporter gene assays[22] and the polymorphism would result in higher constitutive and inducible levels of TNF-$\alpha$. In contrast to a previous work[11] which suggested potential roles of TNF-$\alpha$ 238A in pathogenesis of HBV infection, we failed to find a connection between the TNF-$\alpha$ 238A and chronic HBV infection. Although exact cause can not be explained, geographic and racial difference might play a role in the distribution of allelic polymorphism and immune response. According to our results, the development of HCC seemed not to be related to TNF-$\alpha$ promoter polymorphisms.

Since the TNF-$\alpha$ gene is located very close to the HLA-DR locus, linkage disequilibrium between genetic makers have been described.[13,45-47] We ascertained the role of HLA-DRB1*13 in the self-elimination of HBV in Koreans (Table 4). Furthermore, strong association between TNF-$\alpha$ promoter polymorphism at position -308 and HLA-DRB1*13 suggested a linkage disequilibrium between two alleles. Both TNF-$\alpha$ promoter polymorphism and HLA-DRB1*13 might play a role in the clearance of HBV independently or in a cooperative manner. No association was seen with disease progression and the development of HCC.

MBL is important in the first line of the immune defence because of its ability to act as an acute phase protein and to activate the complement system. Furthermore, MBL could bind to the middle surface protein of the HBV envelope which contains a mannose-rich oligosaccharide.[49] In Asia, including Korea, the most important factor for the persistence of HBV infection is transmission in the neonatal or early childhood period.[50] Thus, MBL seemed to have an important role in protection against HBV. However, earlier studies in this context have provided no conclusive evidence. Some studies failed to detect an association between MBL exon 1 mutations and acute or chronic HBV infection.[51,52] In contrast, other studies showed that the mutation at codon 54 of the MBL gene was associated with progression of disease in chronic HBV and in fulminant hepatitis.[28,53] The mutation at codon 52 of the MBL gene was associated with persistent HBV infection.[27]

As our preliminary study demonstrated that codon 52 and 57 mutations were absent in Koreans unlike to other study.[27] A allele mutation of codon 54 was frequency present in our study.

In our study, codon 52 and 57 mutations were absent, but the A allele mutation of codon 54 was present at high frequency (29.7%), which is in contrast to those of other Asian people, as reported by Thomas *et al.*[27] In addition, we found no significant difference in the frequency of MBL gene mutation in codon 54 between subgroups of HBV infection analyzed. Although the previous work has suggested potential roles of MBL mutation in the progression of HBV infection, our study was unable to reproduce any association between MBL gene mutation and HBV infection.

Interestingly, We found that there was a significant difference in the codon 54 mutation rate between patients with hepatitis B-related HCC and without HCC in CC group (24.2% vs 33.1%, P=0.015) in our study. Whether MBL mutation at codon 54 might be related to the development of HCC or not, further study may be needed.

# V. CONCLUSION

This study implies that variations in the genes governing the level of constitutive and inducible TNF-$\alpha$ are an important factor, which might explain the clearance of HBV infection and offer an approach to elucidating the molecular mechanism of HBV clearance. Substantial genetic association was also noted between TNF-$\alpha$ promoter -308 and HLA-DRB1*13. But MBL gene mutation at codon 54 showed no considerable differences between analyzed group. There were no significant increase in the codon 54 mutation rate in progressive group in Korea HBV patients.

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ABSTRACT(IN KOREAN)

# Tumor necrosis factor-α 의 promoter 부위의 다양성 및 mannose binding lectin gene의 변이와 B형 간염바이러스의 감염경과와의 연관성

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바이러스성 간염의 병리를 밝히기 위한 분자유전학적 연구가 많이 이루어지고 있다. Tumor necrosis factor-α 의 promoter -308 부위의 유전적 다형성이 만성 B형 간염과 관련이 있다는 보고가 있었다. 또한 mannose binding lectin (MBL)의 변이가 혈청내 MBL 농도 감소를 일으켜 면역 결핍으로 인한 만성 B형 간염이 발생한다는 보고도 있었다.

본 연구에서는 염증 반응 및 감염 병원체 제거에 중요한 싸이토카인인 TNF-α 의 발현을 조절하는 것으로 알려진 promoter 부위의 유전자 다형성과 innate immunity에서 중요한 역할을 하고 phagocytosis를 유발하는 MBL의 변이가 다양한 B형 간염 바이러스 감염상태에서 관련이 있는지를 알아보았다.

총 419명의 대상 환자들을 대상으로 하였으며, 이들을 다음과 같이 세 군으로 분류하였다. 1) 비노출군 [89명 HBsAg(-), anti-HBc(-),

anti-HBs(-)], 2) 자연치유군 [85명, HBsAg(-), anti-HBc and anti-HBs(+)], 3) 만성 간염군 [245명, HBsAg(+), anti-HBc(+), anti-HBs(-)]. 만성간염군은 비활동성, 만성 보유자군과 만성 간염, 간경변증, 간세표암을 포함한 진행성 질환군으로 세부 분류하였다. TNF-$\alpha$ 와 MBL은 ABI Prism SNaP shot ddNTP primer extension kit을 사용하여 변이를 관찰 하였다.

자연 치유군 (25.9%) 에서 TNF-$\alpha$ promoter -308부위의 G 에서 A의 전환이 만성 간염군 (6.1%)에 비해 통계적으로 높게 관찰되었다 ($P<0.001$). 한편 TNF-$\alpha$ -238 promoter 의 G에서 A 전환과 MBL codon 54 변이는 각 군간에 유의한 차이를 보이지 않았다.

TNF-$\alpha$ 유전자 promoter -308 부위의 G에서 A 전환이 자연 치유군과 연관성이 있는 것으로 보아 이 부위의 유전자형이 TNF-$\alpha$ 매개기전에 영향을 주며 이것이 B형 간염 바이러스의 제거에 관련이 있을 것으로 생각되어진다. 반면 MBL gene 의 codon 54부위의 변이는 바이러스의 제거 및 질병의 진행성과는 관계가 없는 것으로 생각된다.

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핵심되는 말 : B형 간염 바이러스, tumor necrosis factor (TNF-α), mannose binding lectin (MBL), 유전자 다형성