Relationship between IFN-\(\gamma\) gene polymorphism and susceptibility to intrauterine HBV infection

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AIM: To explore the susceptibility of children to intrauterine HBV infection by studying the relationship between IFN-\(\gamma\) gene polymorphism, including IFN-\(\gamma\)+874A/T single nucleotide polymorphism (SNP) and CA repeat microsatellite polymorphism and intrauterine HBV infection.

METHODS: A TaqMan fluorescence polymerase chain reaction in the IFN-\(\gamma\)+874A/T single nucleotide polymorphism was tested in the intrauterine HBV infection group (group I) and the normal immune children group (group II). Capillary electrophoresis was performed in the above two groups to assay the IFN-\(\gamma\) CA repeat microsatellite polymorphism.

RESULTS: Frequencies of AA, AT and TT genotypes were 67.4%, 19.6% and 13.0% in the intrauterine HBV infection group, and 45.2%, 30.1% and 24.7% in the normal immune children group, respectively. A significant difference was found in the frequency distribution of IFN-\(\gamma\)+874 genotype between the two groups \((\chi^2 = 5.102, P = 0.02389)\). In the intrauterine HBV infection group the AA genotype was more common than in the normal immune group. Frequency of IFN-\(\gamma\)+874A allele was 77.17% in the intrauterine HBV infection group, and 60.27% in the normal immune children group. In the intrauterine HBV infection group the IFN-\(\gamma\)+874A allele was more common than in normal immune group. A significant difference was found in the frequency distribution between the two groups \((\chi^2 = 7.238, P = 0.02389, OR = 2.228, 95\% CI = 1.244-3.992)\).

CONCLUSION: There is a relationship between IFN-\(\gamma\)+874A/T SNP and intrauterine HBV infection as well as between IFN-\(\gamma\) CA microsatellite polymorphism and intrauterine HBV infection. IFN-\(\gamma\) gene polymorphism might be important in determining individual’s susceptibility to intrauterine HBV infection.

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Key words: Interferon-\(\gamma\); Gene polymorphism; Hepatitis B virus; Intrauterine

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INTRODUCTION

IFN-\(\gamma\)+874A/T SNP within the first intron can increase or decrease the binding of a particular transcription factor and ultimately the export of cytokine product\(^{1,2}\). The registered DNA sequences of the human IFN-\(\gamma\) gene deposited in Genbank show the presence of a CA repeat element in the first intron, 875 bp downstream from the start of the first exon. There are seven CA repeats of variable lengths In a consecutive study, 12 CA repeats were confirmed its role as a marker of high IFN-\(\gamma\) production\(^{2}\). We have reported in a previous study that higher IFN-\(\gamma\) secretion than spontaneous secretion could be found in the intrauterine HBV infection group and the normal immune children group with HBsAg stimulation, but the increase in the intrauterine HBV infection group is strikingly lower than that in the normal immune children group\(^{3}\). IFN-\(\gamma\) production is positively associated with IFN-\(\gamma\)-mRNA expression\(^{3}\). In the present study, we studied the IFN-\(\gamma\)+874 SNP and CA repeat microsatellite polymorphism in the intrauterine HBV infection group and the normal immune children group in order to explore the association between IFN-\(\gamma\) gene polymorphisms and susceptibility to intrauterine HBV infection.
MATERIALS AND METHODS

Subjects
The subjects were selected from outpatients who were in our hepatitis B (HB) vaccine follow up clinics. These subjects whose mothers were HBV carriers were inoculated with HB vaccine or HB vaccine plus hepatitis B immunoglobulin (HBIg) and followed up for serum alanine transaminase (ALT) and HBV marker. Intrauterine HBV-infected children were defined based on their positive HBsAg and/or HBV-DNA at birth and lasting for six months (group I). Normal immune children were defined based on their negative HBV marker after birth and high HBsAb titers (group II). ALT of all subjects was normal.

TaqMan fluorescence polymerase chain reaction
Genomic DNA was extracted from peripheral blood using the kits supplied by Sangon Bioengineering Company (Shanghai, China). Polymorphisms at +874 were identified using the ABI Prism 7700 fluorescent automatic sequencer. PCR products were obtained after amplification in a 10 μL volume containing 1× Taqman buffer A, 200 μmol/L dATP, dCTP, dGTP, 400 μmol/L dUTP, 3.5 mmol/L MgCl₂, 300 μmol/L β-actin forward primer, 300 μmol/L β-actin reverse primer, 200 μmol/L β-actin probe, AmpliTaq Gold, 0.025 U/μL DNA polymerase, 0.01 U/μL AmpErase UNG, 900 nmol/L (each) forward & reverse primers, 250 nmol/L TaqMan MGB probe, 20ng DNA template. The forward primer sequence is 5′-ACA TTC CAC AAT TGA TTT TAT TCT TAC AAC A-3′. The reverse primer sequence is 5′-ACG AGC TTT AAA CaC ACA CAC ACA C; Vic: AAA TCA AAT CaC ACA CAC ACA A C. Forty-five cycles of PCR amplification were performed at 50 °C for 2 min, at 95 °C for 10 min, at 95 °C for 30 s and at 60 °C for 30 s.

Capillary electrophoresis
DNA was diluted in a 7.5 μL reaction volume containing 0.75 μL of 10× buffer, 0.75 μL of 25 mmol/L MgCl₂, 0.75 μL of 2.5 mmol/L/LdNTP, 2.5 U/μL Taq Gold, 0.06 μL DNA polymerase, 1 μL (each) forward & reverse primers, 2 μL DNA template, 1.19 μL ddH₂O. The forward primer sequence is CTT CGT TGC TCA CTG GGA TT-6-FAM. The reverse primer sequence is GCA AAG CCA CCC CAC TAT AA. The microsatellite region in the first intron of the IFN-γ gene was amplified on a PTC-100 thermal cycler. Following an initial denaturation (12 min at 95 °C), samples were subjected to 10 cycles of PCR amplification, for each cycle, the sample was amplified at 94 °C for 30 s, at 53 °C for 30 s, at 75 °C for 45 s, followed by 30 cycles at 89 °C for 30 s, at 53 °C for 30 s, at 75 °C for 45 s, and a final extension at 4 °C. The IFN-γ CA repeat microsatellite polymorphisms were monitored by ABI PRISM 3700 capillary electrophoresis automatic analysis.

Statistical analysis
IFN-γ+874A/T allele frequencies and IFN-γ CA microsatellite frequencies were evaluated by gene count. Allelic frequency was calculated with the following equation: allelic frequency = n/2N (n represents the number of alleles, and N represents the total number of subjects). Allelic frequencies were compared using the chi-square test. A P value less than 0.05 was considered statistically significant. The strength of an association was expressed as odds ratio (OR) with 95% confidence interval. STATA 6.0 software was used to determine the statistical significance.

RESULTS

General clinical characteristics
No significant difference was found in sex, HBeAg positivity of mother and HBIg injection to pregnant women between the two groups (Table 1).

IFN-γ+874A/T SNP
The distribution of IFN-γ+874A/T allele frequencies in two groups was in accordance with Hardy-Weinberg hereditary equilibrium law. Frequencies of AA, AT and TT genotypes were 67.4%, 19.6% and 13.0% in the intrauterine HBV infection group and 45.2%, 30.1% and 24.7% in the normal immune children group, respectively. A significant difference was found in the frequency distribution of IFN-γ+874T genotype between the two groups (χ² = 5.102, P = 0.02389). In the intrauterine HBV infection group, the AA genotype was more common than in normal immune group (Table 2). In the intrauterine HBV infection group, the AA genotype was more common than in normal immune group.

Statistical analysis
IFN-γ+874A allele and IFN-γ+874T allele frequencies were calculated with the following equation: allelic frequency = n/2N (n represents the number of alleles, and N represents the total number of subjects). Allelic frequencies were compared using the chi-square test. A P value less than 0.05 was considered statistically significant. The strength of an association was expressed as odds ratio (OR) with 95% confidence interval. STATA 6.0 software was used to determine the statistical significance.

Table 1  Clinical characteristics of patients and controls

| Subjects | n | Sex | HBV marker of mother | HBIg injection in pregnancy |
|----------|---|-----|----------------------|----------------------------|
| Group I  | 46 | 26  | 20                   | 15                         | 31                         | 10  | 30  |
| Group II | 73 | 41  | 32                   | 25                         | 48                         | 16  | 47  |

Z² = 7.238, P = 0.02389, OR = 2.228, 95% CI 1.244-3.992 (Table 3).

Group I: the intrauterine HBV infection group; Group II: the normal immune children group.

Table 2  Frequencies of IFN-γ+874 genotype in patients and controls

| Subjects | n | Number of subjects and percentage of IFN-γ+874 genotype (%) |
|----------|---|------------------------------------------------------------|
|          | AA | AT | TT |
| Group I  | 46 | 31 (67.4) | 9 (19.6) | 6 (13.0) |
| Group II | 73 | 33 (45.2) | 22 (30.1) | 18 (24.7) |

Z² = 5.102, P = 0.02389

Group I: the intrauterine HBV infection group; Group II: the normal immune children group.

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Table 3  +874T and +874A IFN-γ allele frequencies in patients and controls

| Allele (n) | IFN-γ+874A/T allele frequencies (%) | OR | 95% confidence interval | χ² | P |
|-----------|-------------------------------------|----|-------------------------|----|---|
| +874A     | +874T                               |    |                         |    |   |
| Group I:  |                                     |    |                         |    |   |
| 46 (92)   | 71 (77.17)                          | 12.94-3.992 | 7.238 0.0071 |    |   |
| Group II: |                                     |    |                         |    |   |
| 73 (146)  | 88 (60.27)                          | 58 (39.73) |    |    |   |

Group I: the intrauterine HBV infection group; Group II: the normal immune children group.

Table 4  IFN-γ CA microsatellite morphisms in patients and controls

| n | Number and percentage of genotype of IFN-γ CA (%) |
|---|-----------------------------------------------|
|   | (CA0)²/(CA0)² | (CA0)/(CA0)² | (CA0)/(CA0)² |
| Group I: 42 | 9 (11.80) | 11 (26.19) | 26 (61.91) |
| Group II: 68 | 32 (26.47) | 23 (33.82) | 27 (39.71) |
| χ² | 5.640 |
| P  | 0.0176 |

Group I: the intrauterine HBV infection group; Group II: the normal immune children group.

**IFN-γ CA microsatellite polymorphism**

(CA0)²/(CA0)² of IFN-γ CA microsatellite polymorphism was 11.90% in the intrauterine HBV infection group and 26.47% in the normal immune children group. A significant difference was found in the frequency distribution between the two groups (χ² = 5.64, P = 0.0176)(Table 4). Frequency of IFN-γ CA repeat was 25% in the intrauterine HBV infection group and 43.38% in the normal immune children group. In the intrauterine HBV infection group, the frequency of IFN-γ CA repeat was less than in normal immune group. A significant difference was found in the frequency distribution between the two groups (χ² = 7.548, P = 0.0060)(Table 5, Figure 1).

**DISCUSSION**

The clinical features of HBV infection depend on the activities of the host immune response and the virus. The cell-mediated immune response to HBV-encoded antigen is responsible both for viral clearance and for disease pathogenesis during this infection. T-helper lymphocyte response mediated through secretion of IFN-γ plays an important role in inducing viral clearance[1]. During the early specific phase of host defense, production of IFN-γ by natural killer(NK) cells plays an important role in bringing about acute inflammation. In the subsequent antigen-specific phase of the immune response, IFN-γ acts as a regulator of antigen presentation and proliferation as well as differentiation of lymphocytes. High-level production of IFN-γ during this phase plays an important role in viral clearance[2].

Cytokine production is genetically controlled. The cytokine genes SNPs in cis-acting regions can alter transcriptional activity and are associated with the production of cytokine[3]. Genetic susceptibility to diseases is likely influenced by common DNA variants in the form of SNPs[4-5]. Pravica et al[6] described a variable length CA repeat sequence in the first intron of the human IFN-γ gene and showed that 12 CA repeat is associated with high *in vitro* IFN-γ production. In a further study, a SNP T to A at the end of the CA repeat region in the first intron of the human IFN-γ gene(+)874A/T was described, showing an absolute correlation between the T allele and the high IFN-γ microsatellite polymorphism. The +874T allele is associated with the high production of genotype and the +874A allele is associated with the low production of genotype[7]. *In vitro* production of IFN-γ has a significant correlation with the CA microsatellite polymorphism. Twelve CA repeats have been shown to have a higher expression of IFN-γ[8-12]. Japanese researchers[13] have reported the frequencies of 13 CA repeats are significantly greater in patients with IgA nephropathy than in the healthy control group (43% versus 23%, P<0.05). High production of genotype (12 CA repeats) for IFN-γ may have an influence on acute rejection of kidney transplant[14]. The frequency of the IFN-γ12 CA repeats is significantly greater in WHO class V lupus nephritis patients than in WHO class IV patients[15]. Susceptibility to and severity of rheumatoid arthritis are related to a microsatellite polymorphism.

![Figure 1 IFN-γ CA microsatellite polymorphisms in patients and controls. Group I: the intrauterine HBV infection group; Group II: the normal immune children group.](www.wjgnet.com)
within the first intron of the IFN-γ gene\(^\text{[14]}\). There is a highly significant increase in the low-production of IFN-γ genotype (13 CA repeats) in patients with type 1 diabetes compared with normal healthy controls\(^\text{[15]}\). This result suggests that polymorphisms of the IFN-γ gene may modify the function of this proinflammatory mediator and the response to pancreatic islet \(\beta\) cells. This \(+874\)A/T polymorphism coincides with a putative NF-Kappa B binding site, which might have functional consequences for transcription of human IFN-γ gene. Therefore, the T to A polymorphism might directly influence the level of IFN-γ production associated with the CA microsatellite polymorphism\(^\text{[14]}\). Patients with tuberculosis have a lower frequency of \(+874\)TT genotype than the controls, suggesting that genetically determined variability in IFN-γ and expression might be important for the development of tuberculosis\(^\text{[10]}\). A significant correlation has been detected between the presence of high-expression polymorphisms of the IFN-γ genes and bronchiolitis obliterans syndrome (BOS) after lung transplantation\((P = 0.039)\). \(+874\)TT of the IFN-γ gene significantly increases the risk of BOS after lung transplantation\(^\text{[20]}\). There is no report on the relationship between IFN-γ gene polymorphism and susceptibility to intrauterine HBV infection.

In this study, the frequency of the \(+874\)AA genotype of the IFN-γ was greater in the intrauterine HBV infection group than in normal immune group, the \((\text{CA})_0^+/\text{(CA})_0^+\) of IFN-γ CA microsatellite polymorphism was 11.90% in the intrauterine HBV infection group and 26.47% in the normal immune children group. A significant difference was found in the frequency distribution between the two groups\((\chi^2 = 5.640, P = 0.0176)\). The frequency of IFN-γ CA repeat was less in the intrauterine HBV infection group than in the normal immune group. A significant difference was found in the frequency distribution between the two groups\((\chi^2 = 7.548, P = 0.0060)\). The findings suggest that the presence of low-expression polymorphism at \(+874\) of the IFN-γ gene significantly increases susceptibility to intrauterine HBV infection. IFN-γ gene polymorphism might be important in determining an individual’s susceptibility to intrauterine HBV infection and might result in viral persistence.

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