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Interferon-Gamma Gene Polymorphism +874 (A/T) in Chinese Children with Henoch-Schönlein Purpura

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ABSTRACT

The aim of this study was to investigate the possible influence of Interferon-gamma (IFN-γ) gene polymorphism +874 (A/T) (rs2430561) in the susceptibility and renal complications of patients with Henoch-Schonlein purpura (HSP). We also studied the effects of IFN-γ allelic variation on serum levels of pro-and anti-inflammatory cytokines in HSP patients.

The study population comprised 97 patients suffering from HSP and 97 control participants. Patients and controls were genotyped for a single nucleotide polymorphism +874 (A/T) in the first intron of the IFN-γ gene by the TaqMan PCR method.

Frequencies of individuals with IFN-γ +874 AA, AT and TT genotypes were 77.3%, 21.6% and 1% in HSP patients and 79.4%, 17.5% and 3.1% in controls, respectively. The frequency of the AA genotype in HSP patients with nephritis was slightly higher (83.3%) than in HSP patients without nephritis (73.8%). The allele A occurred more commonly in HSP patients with nephritis (92%) than in HSP patients without nephritis (86%), but these differences were not statistically significant (p= 0.469 and p= 0.244, respectively). In addition, significant difference in serum IL-10 levels between IFN-γ +874 different genotype groups was found.

Our results do not support a role for IFN-γ gene polymorphism +874 (A/T) in the susceptibility to HSP and allelic variation at IFN-γ +874 locus had no effect on serum levels of cytokines in patients with HSP except for IL-10.

Keywords: Cytokines; Henoch-Schonlein purpura; Interferon-gamma; Polymorphism; Susceptibility

INTRODUCTION

Henoch-Schonlein purpura (HSP) is small vessel vasculitis that occurs mainly in childhood,[1] with an incidence of 10–20 per 100,000 children annually,[2] characterized pathologically by immunoglobulin A (IgA) dominant immune deposition in the small vessels.[3] It occurs most frequently in children between the ages of 2 and 11 years.[4] HSP is generally an acute and self-limited condition that lasts an average of 4 weeks. Purpura of the skin, joint pain and swelling,

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gastrointestinal symptoms, and renal involvement are the most common manifestations of the patient population. Nephritis is one feature of HSP that may have chronic consequences, and the long-term prognosis is heavily dependent on the severity of nephritis.

Acute inflammation in HSP manifests as leukocytoclastic vasculitis on light microscopy. Although the pathogenesis of HSP has not been fully elucidated, experimental evidence suggests inflammatory cytokines such as IL-1, TNF and IL-6 are involved in the pathogenesis of HSP.[5]

The inflammatory response is modulated by the balance between pro-inflammatory and anti-inflammatory mediators.[6] Interferon- gamma (IFN-γ) derived from activated T-helper 1 (Th1) cells is a pro-inflammatory cytokine and plays a pivotal role in the initiation, regulation, and maintenance of the inflammatory response. This cytokine has the potential to direct the inflammatory response by upregulating a variety of pro-inflammatory mediators including TNF-α and IL-6.[7] Moreover, data suggest that IFN-γ may also be able to directly enhance activation of the pro-inflammatory nuclear transcription factor-κB (NF-κB) under certain conditions.[8] According to these functional characteristics, IFN-γ bioactivity has been identified as a key mediator in several models of inflammatory diseases.[7]

IFN-γ is encoded by a single gene mapped on chromosome 12 (12q15).[9] In the first intron of the IFN-γ gene, there is a CA repeat polymorphism that affects transcription. Moreover, an adenine (A) to thymine (T) transition at position +874 (rs2430561) has been associated with increased IFN-γ expression.[9] The transcription factor NF-κB binds preferentially to the +874T allele.[8] This preferential binding suggests that genetically determined variability in IFN-γ expression might be important for the inflammatory response activated through the NF-κB pathway. IFN-γ gene+874 (A/T) polymorphisms have been shown to be associated with several rheumatic autoimmune diseases, such as systemic lupus erythematosus,[10,11] multiple sclerosis[12], Wegener's granulomatosis (WG)[13] and rheumatic fever.[14]

The aim of this study was to assess whether the IFN-γ gene polymorphism +874 (A/T) is associated with susceptibility and renal complications of patients with HSP. We also studied the effects of IFN-γ +874 genotypes on serum levels of IFN-γ and of other pro- and anti-inflammatory cytokines in HSP patients**.**

MATERIALS AND METHODS

Patients and Controls

Ninety-seven children with HSP during June 2011 and May 2012 were recruited from the Children's Hospital of Medical College, Zhejiang University, China. Fifty-five subjects were male (mean age: 6.5±2.2 years) and 42 were female (mean age: 7.6±2.8 years). All cases fulfilled the American College of Rheumatology (ACR) criteria for the classification of HSP.[15] All patients who had been newly diagnosed with HSP and that had been on follow-up for at least six months were included in this study.

All 97 children had typical skin purpura and/or maculopapular rash, 57 children (58.8%) had arthritis or arthralgia, 71 children (73.2%) suffered from abdominal pain, 36 children (37.1%) had renal complications, and one child (1.0%) appeared to have renal insufficiency.

Renal needle biopsies were performed on eighteen cases in the HSP nephritis group, of which nine cases were grade II, eight grades III and one grade IV. The diagnosis was based on the criteria established by the International Study of Kidney Diseases in Children (ISKDC).[16]

Ninety-seven healthy children without a history of allergic purpura were randomly selected to be enrolled in the study, of which 56 were male (mean age: 6.9 ± 2.0 years) and 41 were female (mean age: 7.0 ± 2.1 years).

All subjects were of Han origin from Zhejiang province in eastern China. Informed consent was obtained from all the families of the patients and controls, and the study was performed with the hospital Ethical Committee approval.

The following variables were determined for HSP patients from the hospital records: Gender, present age, age at diagnosis, inflammatory activity (erythrocyte sedimentation rate, C-reactive protein, leukocyte count, cytokines), serum levels of creatinine, albumin, C3, C4, IgA, IgE and proteinuria.

Clinical Definitions

Nephritis was defined as the presence of any haematuria or proteinuria. Hematuria was defined as more than 3 red blood cells per high-power field in

urine sediment. Proteinuria was defined as a 24-hour urine collection containing more than 150 mg of protein.[17] Hypertension was defined as blood pressure higher than the 95th percentile for age according to data from Task Force Report on High Blood Pressure in Children and Adolescents. Renal insufficiency was considered if the plasma creatinine concentration was above the upper limit of normal.

Analysis of IFN- γ Gene Polymorphism

Leukocytes were withdrawn by centrifugation from anticoagulated blood samples. DNA of the leukocyte genome was extracted using a standard proteinase K digestion and phenol-chloroform method. Genotyping was performed with the TaqMan SNP genotyping assay using the LightCycler 480 II Real-Time PCR System (Roche Applied Science, Switzerland). The primers and TaqMan probe sequences were:

rs2430561-F：

5′-ATAGTTCCAAACATGTGCGAG-3′;

rs2430561-R：

5′-TTCAGACATTCACAATTGATTTTA-3′;

rs2430561- Probe T allele：

5′-FAM-CAAAATCAAATCTCAC-MGB-3′;

rs2430561- Probe A allele：

5′-VIC-CAAAATCAAATCACAC-MGB-3′.

TaqMan PCR reaction system was 20μl, including: genotype qPCR Master Mix (2×) 10 μl, PCR forward primer (500nM) 1μl, PCR reverse primer (500nM) 1μl, TaqMan probe T (250nM) 0.5μl, TaqMan probe C (250nM) 0.5μl, genomic DNA 1μl (100ng), dH_2O 6μl. We followed the instructions provided with the assay kit (Huirui Biotechnology Co, Ltd, Shanghai, China). Briefly, the run consisted of a hot start DNA polymerase at 95°C for 5 minutes, followed by 40 cycles of denaturing at 95°C for 10 seconds and annealing at 55°C for 45 seconds. Deionized water was used as the negative control. About 10% of the samples were randomly selected for repeated genotyping for confirmation, and the results were 100% concordant.

Statistical Analysis

Statistical analysis was performed using the SPSS software package, revision 13.0. Serum IFN-γ, TNF-α, IL-2, IL-4, IL-6 and IL-10 levels were presented as the mean ± standard deviation (SD). The Hardy–Weinberg equilibrium was assessed using chi-square test based on comparison of the observed and expected genotypes. Differences in the distributions of IFN-γ genotypic and allelic frequencies between the groups were tested using the chi-square test. Mean values were compared between the groups by the unpaired student's t-test. The differences between the groups were considered significant if *p*-values were <0.05.

RESULTS

Ninety-seven HSP patients and ninety-seven controls from eastern China were studied. The main clinical characteristics and laboratory parameters of the patients with HSP are shown in Table 1.

The frequencies of genotypes and alleles at position +874 of IFN-γ are shown, respectively, for HSP patients, controls and HSP stratified by the presence of nephritis in Table 2. There was no evidence of departure from the Hardy-Weinberg equilibrium in the HSP patients and controls (χ^2= 0.12 and 2.53, *p*= 0.729 and 0.112, respectively).

The distributions of IFN-γ genotypic and allelic frequencies were not significantly different in the HSP

Table 1. The clinical and laboratory characteristics of children with HSP at the time of diagnosis

Clinical and laboratory characters	Patient group, n (%),or mean ± SD
Age at disease onset, yrs	7.0±2.5
Range	2-14
Gender (male/female)	55/42
Purpuric rash	97(100)
Arthralgia and/or arthritis	57(58.8)
Gastrointestinal manifestations	71(73.2)
Bleeding	26 (26.8)
Bowel angina	71 (73.2)
Renal involvement	36(37.1)
Hematuria	36 (37.1)
Proteinuria	31 (32.0)
Renal insufficiency	1 (1.0)
Erythrocyte sedimentation rate(mm/h)	18.10±19.21
White blood cell ($\times10^9$/L)	10.44±5.19
C-reactive protein (mg/l)	8.59±15.88
Serum creatinine (mg/dl)	0.49±0.23
C3 (g/l)	1.20±0.24
C4 (g/l)	0.31±0.24
IgA (g/l)	1.97±0.83
IgE (IU/ml)	193±327

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Table 2. Distributions of IFN-γ(+784) genotypic and allelic frequencies in HSP patients and healthy controls

Gene	Genotype/ allele	Controls (n=97)	HSP (n=97)	*P*[a]	HSP without nephritis (n=61)	HSP with nephritis (n=36)	*P*[b]
IFN-γ(+784) rs2430561	A/A	77 (79.4%)	75 (77.3%)	0.485	45(73.8%)	30 (83.3%)	0.469
	T/A	17 (17.5%)	21 (21.6%)		15(24.6%)	6 (16.7%)	
	T/T	3 (3.1%)	1 (1%)		1(1.6%)	0 (0%)	
	Allele A	88%	88%	1	86%	92%	0.244
	Allele T	12%	12%		14%	8%	

[a] Total HSP patients vs. controls ; [b] HSP without nephritis vs. HSP with nephritis

Table 3. The serum levels of cytokines in HSP children with IFN-γ (+784) AA genotype and non-AA (AT+TT) genotype

	AA genotype n=59	Non-AA genotype n=19	*P*-value
IFN-γ (pg/ml)	3.36±1.20	2.96±0.97	0.186
TNF-α (pg/ml)	3.40±5.45	2.68±0.48	0.566
IL-2 (pg/ml)	1.86±2.10	1.59±0.54	0.580
IL-4 (pg/ml)	2.16±0.72	2.00±0.53	0.351
IL-6 (pg/ml)	7.80±10.77	5.10±3.52	0.287
IL-10 (pg/ml)	3.73±2.63	2.83±0.76	0.022

Values are expressed as the mean± SD

patients and the control group (Table 2). Frequencies of individuals with IFN-γ +874 AA, AT and TT genotypes were 77.3%, 21.6% and 1% in HSP patients and 79.4%, 17.5% and 3.1% in controls, respectively.

In addition, the allelic and genotypic frequencies were also examined in HSP patients stratified by the presence of nephritis during the course of the disease. The frequency of the AA genotype in HSP patients with nephritis was slightly higher (83.3%) than in HSP patients without nephritis (73.8%). The allele A occurred more commonly in HSP patients with nephritis (92%) than in HSP patients without nephritis (86%), but these differences were not statistically significant (p=0.469 and p=0.244, respectively).

The serum IFN-γ, TNF-α, IL-2, IL-4 and IL-6 levels in the acute stage of HSP were not statistically different between patients with AA genotype and Non-AA genotype (AT and TT). The serum IL-10 levels were significantly higher in patients with the AA genotype than that in the non-AA group (3.73±2.63 vs.2.83±0.76, p= 0.022) (Table 3). Of 36 patients with nephritis, 30 had the AA genotype, 6 had the AT genotype, and none had the TT genotype.

DISCUSSION

Although HSP has been suggested to represent an IgA related immune complex disease, the pathogenesis has not been fully elucidated.[18] A number of factors may contribute to the development of HSP. In addition to infection as a possible trigger factor, genetic predisposition is considered to be associated with HSP.

IFN-γ polymorphism is a good candidate to be evaluated in inflammatory diseases as it is a principal mediator of innate as well as adaptive immunity in the defense against bacteria, viruses and fungi. To our knowledge, this study is the first to examine the potential role of IFN-γ gene polymorphism +874 (A/T) in the susceptibility to patients with HSP. Previous studies examined the possible role of the polymorphism IFN-γ +874 (A/T) in human IgA nephropathy (IgAN) and WG. A family-based association study from Italy[19] demonstrated that the +874A allele confers susceptibility to IgAN and does not influence renal survival. Spriewald et al.[13] reported that a higher frequency of the IFN-γ+874 TT genotype was found in WG patients than in controls (34% versus 14%, OR= 3.14) and the IFN-γ +874 AA genotype was more

prevalent in the end stage renal disease (ESRD) subgroup. They concluded that distinct IFN-γ polymorphisms are associated with susceptibility and outcome in generalised WG. However, in the cohort of HSP patients in the present study, the frequency of genotypes in the IFN-γ + 874 is similar to that observed in the normal control population. Hence, we did not detect the association of IFN-γ gene polymorphism +874 (A/T) with the susceptibility to HSP, and this was also the case when HSP patients were stratified by the presence of renal manifestations.

The mechanisms that control the production of IFN-γ have been studied. The association of +874 alleles A to T with a low (AA), medium (AT) and high (TT) cytokine production was shown in vitro.[20] We studied the effect of IFN-γ gene polymorphism +874 (A/T) on serum levels of several pro- and anti-inflammatory cytokines including IFN-γ in patients with HSP. In previous studies, the TT "high producer" genotype thought to be associated with higher IFN-γ concentration or higher IFN-γ expression was reported in atopic[21] and haemodialysis patients,[22] respectively. Nevertheless, we cannot confirm this finding in our present study. Our data showed that the IFN-γ +874 AA genotypes were associated with a slightly higher serum IFN-γ concentration compared to non-AA genotypes, however, the difference did not reach a statistical significance (3.36±1.20 vs. 2.96±0.97, p=0.186). The frequency of mutant homozygote (TT) was low in our study, thus studies with larger sample sizes are required to confirm this observation. Additionally, we did not observe any significant effects of IFN-γ gene polymorphism +874A/T on other cytokines besides IL-10.

The serum IL-10 levels were significantly higher in patients with the IFN-γ +874 AA genotype. IL-10 inhibits the production of pro-inflammatory cytokines by inhibition of Th1 lymphocytes and stimulation of B lymphocytes and Th2 lymphocytes and thus down-regulates the inflammatory response.[23-25] Thus, we deduced that IL-10 may play a role in counterbalancing the effects of pro-inflammatory cytokines such as IFN-γ, IL-2, IL-6 and TNF-α in the pathogenesis of HSP.

Our data suggest that IFN-γ gene polymorphism +874 (A/T) does not seem to be associated with susceptibility to HSP in a Chinese population. IFN-γ +874 genotypes have no relationship with serum cytokines levels except IL-10. Further studies with a larger sample size are needed to confirm the present findings.

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