Genes and Immunity (2001) 2, 357–362

www.nature.com/gene

Interleukin 6 gene promoter polymorphism is not associated with Kawasaki disease

MH Sohn[1], M-W Hur[2] and DS Kim[1]
[1]*Department of Pediatrics, and* [2]*Biochemistry and Molecular Biology, Yonsei University College of Medicine, Severance Hospital, CPO Box 8044, Seoul 120–752, Korea*

We examined the IL-6 gene promoter and detected several interesting promoter polymorphisms: GGGCTG insertion at +162 bp and G deletion at +168 bp positions (M1), A to G substitution at −594 bp (M2) of the reported IL-6 promoter sequence. Other rare variations were also observed at several positions: −583 bp (T insertion), −507 bp (C insertion), −71 bp (T deletion), +17 bp (C insertion), and +121 bp (GC insertion). Although Kawasaki disease (KD) patients demonstrate a drastic increase in serum interleukin-6 (IL-6) during the acute phase that parallels the duration of fever, there were no significant differences in the nucleotide sequence between the KD patients and normal control group. By transient transfection with IL-6 gene promoter-luciferase fusion plasmids into CV-1 cells, we tested the functional significances of the polymorphisms. Mutations at +162 bp, +168 bp and −594 bp significantly decreased luciferase expression ($P < 0.05$), suggesting the promoter elements flanking the mutated nucleotides are important in transcriptional activation. Genes and Immunity (2001) **2,** 357–362.

Keywords: *Kawasaki disease; IL-6, promoter; polymorphism*

Introduction

Kawasaki disease (KD) is an acute febrile illness that primarily affects infants and young children.[1] The major clinical features are fever lasting longer than 5 days, bilateral conjunctival injection, enlarged cervical lymph nodes, erythematous indurations of hands and feet, inflammation of lips, oropharynx, and tongue, and polymorphous skin rashes.[2]

Despite well-defined clinical descriptions, its underlying pathogenesis and the cause of the disease remain to be elucidated. Many studies have suggested that immune activation may contribute to the pathogenesis of KD. Abnormalities of immunoregulation include increased in activated $CD4^+$ T cells[3] and activated monocytes,[4,5] and polyclonal activation of B cells.[6–8] In addition, inflammatory cytokines such as tumor necrosis factor-α (TNF-α),[9] interleukin-1β (IL-1β),[10] and interleukin-6 (IL-6)[11,12] have been shown to be elevated in the serum of the Kawasaki patients during the acute phase.

IL-6 is a pleiotropic cytokine involved in the regulation of immune response, acute phase reaction and hematopoiesis. IL-6 is produced by a variety of cells after various stimulations such as infection, trauma and immunological challenge.[13] Serum IL-6 rises significantly in conjunction with fever during acute phase, and then falls to normal in parallel with return of body temperature during subacute phase of the KD.[11] Interestingly, serum IL-6 during acute phase showed a significant correlation with the duration of fever in the patients who were not treated with intravenously administered gamma globulin (IVGG).[14] Thrombocytosis is frequently observed during subacute phase of disease, which might be caused by stimulation of IL-6 to megakaryocytes in bone marrow. Many of the clinical and laboratory features of KD are a reflection of a vigorous hepatic acute phase response, mainly caused by IL-6.[12]

These findings may suggest that the regulation of IL-6 expression in KD patients differs from normal children, possibly by promoter polymorphisms. In Caucasians, two of the point mutations have been described: the G/C element at −172 bp[15] and the G/A element at −594 bp.[16] In Japanese woman, the C/G substitution at −634 bp and the G/A substitution at 4391 in 3′ non-coding portion of exon 5 were identified.[17] A biallelic (G/C) single nucleotide polymorphism (SNP) at −573 bp was identified in African-Americans.[18] Recently Jordanides *et al*[19] examined three SNP at positions −594, −570, −192 in the 5′ promoter region and one VNTR at the 3′ region in the human IL-6.

In relation to the disease, it was reported that the systemic lupus erythematosus (SLE)-associated IL-6 XbaI restriction alleles had duplications of AT-repeat sequences and this variability may be related to the aberrant IL-6 expression.[20] In the sporadic Alzheimer's disease, an association of the C allele of the IL-6 genotypes with delayed initial onset and reduced disease risk was noted.[21] Fishman *et al*[22] reported that G to C mutation at −174 bp in the IL-6 promoter of the patients with systemic-onset juvenile chronic arthritis. The −174 C allele showed lower IL-6 expression than the −174 G

Correspondence: Dr Dong Soo Kim, Department of Pediatrics and Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Severance Hospital, CPO Box 8044, Seoul 120-752, Korea. E-mail: dskim6634@yumc.yonsei.ac.kr
This work was partially supported by the Pediatric Fund of YUMC (JS Kim, HK Lee).
MH Sohn and M-W Hur contributed equally to this work.
Received 14 March 2001; revised 15 May 2001; accepted 2 July 2001

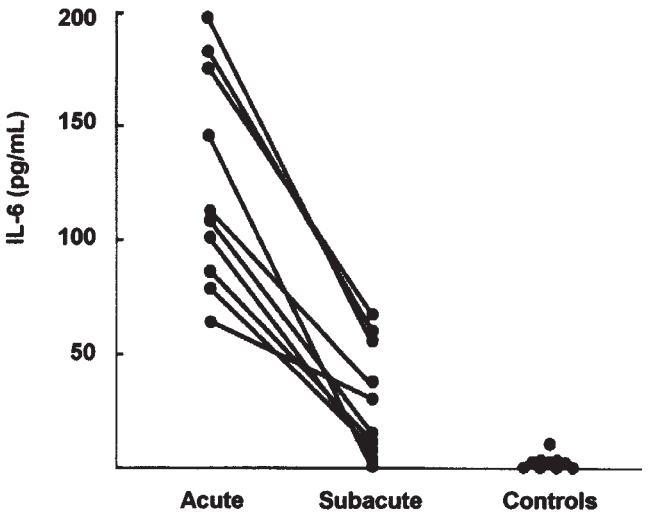


Figure 1 Serum interleukin-6 levels in serial samples obtained from the Kawasaki patients during acute and subacute phases of illness and normal controls.

allele. IL-6 serum levels are lower in subjects with the CC genotype compared with GC or GG subjects. These findings suggest that the individual IL-6 genotype may be relevant in other conditions where IL-6 levels are increased, as in KD.

With these considerations, we investigated the IL-6 promoter polymorphism in KD patients and healthy controls to explore the possibility that IL-6 gene may be involved in the pathogenesis of KD.

Results

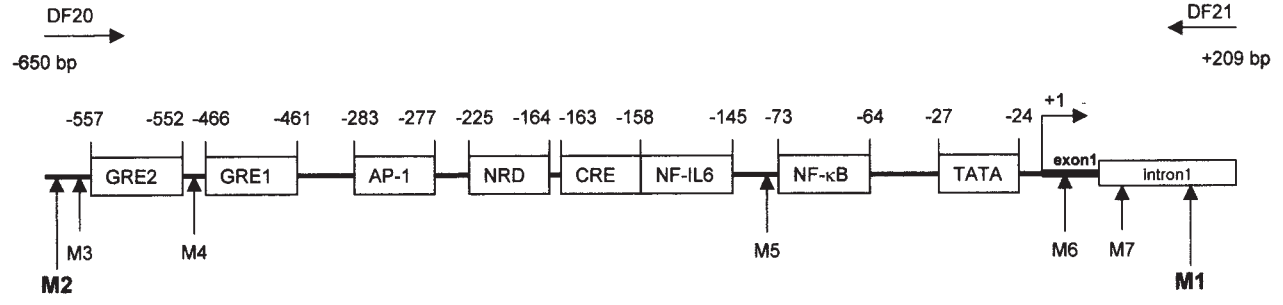
Serum IL-6 levels

In all 10 healthy children, serum IL-6 level was below 5 pg/ml. In contrast, the serum IL-6 level was markedly elevated in patients with acute KD, ranging from 60.5 to 208.3 pg/ml. The mean value was 136.9 pg/ml. As shown in Figure 1, IL-6 level in sera collected during subacute phase was significantly reduced compared with that during acute phase of KD which ranged from undetectable to 72.5 pg/ml: the mean was 20.9 pg/ml.

Polymorphism of IL-6 promoter

G to C substitution polymorphism at –174 bp was not found in KD patients and normal control. However, we

a



b

	Region (bp)	Wild	Mutation	
Frequent polymorphism				
M1	+162, +168	5'-GA***T***GCGGG***G***CG-3'	5'-GA***(GGGCTG)***GCGGG***(-G)***CG-3'	Insertion and Deletion
M2	-594	5'-TGAGG***A***TGGCC-3'	5'-TGAGG ***(G)*** TGGCC-3'	Substitution
Rare polymorphism				
M3	-583	5'-GGCA***G***TCTA-3'	5'-GGCAG ***(T)*** TCTA-3'	Insertion
M4	-507	5'-ATAA***T***CCAG-3'	5'-ATAAT***(C)***CCAG-3'	Insertion
M5	-71	5'-ATGT***G***GGAT-3'	5'-ATGT***(-G)***GGAT-3'	Deletion
M6	+17	5'-CGAG***C***CACC-3'	5'-CGAGC***(C)***CACC-3'	Insertion
M7	+121	5'-CAGC***C***GGTC-3'	5'-CAGCC***(GC)***GGTC-3'	Insertion

Figure 2 (**a**) A schematic representation of the 5′ flanking region of the IL-6 gene indentifying the location of mutation and transcription factor binding sites: GRE1 and GRE2 (glucocorticoid responsive elements 1 and 2), AP-1 (activator protein-1), NRD (negative regulatory domain), CRE (cAMP responsive element), NF-IL6 (nuclear factor of interleukin-6), NF-κB (nuclear factor kappa B); (**b**) Regions and sequences of mutation compared with wild-type IL-6 promoter.[20]

detected several interesting promoter polymorphisms: GGGCTG insertion at +162 bp and G deletion at +168 bp (M1), A to G substitution at –594 bp (M2) of the reported IL-6 promoter sequence (Figure 2). The M1 and M2 mutations were seen simultaneously in seven of 10 KD patients and also in seven of 10 normal controls. Other rare consistent sequence variations different from the reported promoter sequence were observed: –583 bp (T insertion), –507 bp (C insertion), –71 bp (T deletion), +17 bp (C insertion), and +121 bp (GC insertion). There were no significant differences between the KD patients and normal control group.

Functional studies

We suspect that M1 and M2 mutation might have a functional influence on the expression of IL-6 gene. We introduced the IL-6 promoter-luciferase constructs containing M1 and M2 polymorphism into CV-1 and HeLa cells by transient transfection (Figure 3). In unstimulated CV-1 cells, the M1 construct showed 1.5-fold lower expression than the wild-type. M2 construct showed 2.2-fold lower expression than wild-type. The plasmid bearing M1 and M2 mutation showed the lowest expression and 6.2-fold lower expression than wild-type ($P < 0.05$). Upon stimulation with IL-1, expression from M1 construct increased respectively compared with the wild-type. M2 construct increased 1.8-fold compared with M1 construct. The plasmid with M1 and M2 mutations increased 3.3-fold compared with wild-type ($P < 0.05$). On stimulation with lipopolysaccharide (LPS), expression from M1 construct increased 1.8-fold compared with the wild-type, M2 construct increased 2.4-fold compared with wild-type. The plasmid with M1 and M2 mutations increased 5.9-fold compared with wild-type ($P < 0.05$). When HeLa cells were stimulated with IL-1 or LPS, no significant increase was noted compared to the results using CV-1 cells. Only a significant difference was noted in the expression of IL-6 gene between the wild-type and the plasmid with M1 and M2 mutations in the unstimulated cells ($P < 0.05$).

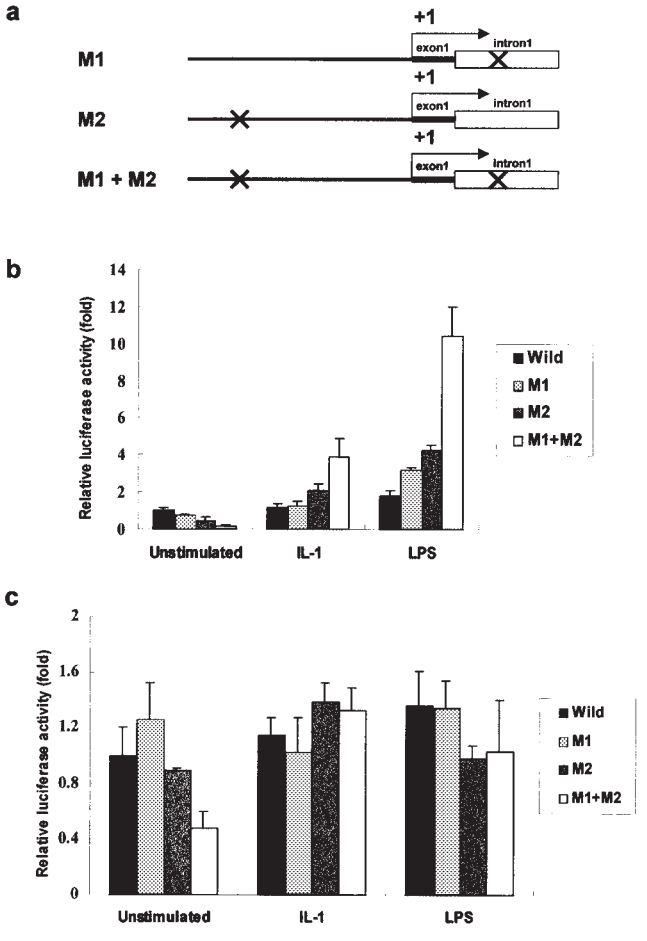


Figure 3 (**a**) A schematic representation of the plasmid containing M1, M2 mutations used for transient transfection assays: M1 (IL-6 promoter luciferase fusion construct with mutation at +162 bp and +168 bp), M2 (IL-6 promoter luciferase fusion construct with mutation at –594 bp), M1+M2 (IL-6 promoter luciferase fusion construct with M1 and M2 mutation), **X** (site of mutation identified), +1 (transcription start site), → (direction of transcription); (**b**) Luciferase activity expressed in CV-1 cells. M1 and M2 mutations significantly decreased luciferase expression in unstimulated cells and significantly increased luciferase expression by stimulation with either IL-1 or LPS; (**c**) Luciferase activity expressed in HeLa cells. A significant difference in reporter expression was noted only in the double mutant (M1 and M2) construct in unstimulated cells.

Discussion

In contrast to our expectation, there was no evidence for genetic association conferred by IL-6 promoter polymorphisms at the position –174 with respect to susceptibility to KD. Interestingly, there is another report that IL-6 promoter polymorphisms at –174 region is not associated with ankylosing spondylitis,[23] even though IL-6 levels increased during this disease. This potentially suggest the difference in post-transcriptional regulation of IL-6 gene expression between normal group and KD patients.

And there were no significant differences in the nucleotide sequences between the KD patients and normal control groups. However, we detected several interesting promoter polymorphisms both in KD and normal controls: GGGCTG insertion at +162 bp, G deletion at +168 bp positions (M1) and A to G substitution at –594 bp (M2) of the reported IL-6 gene promoter sequence. Especially –594 position was the same site reported as FokI polymorphism by Faulds *et al*.[16] And other rare consistent sequence variations different from the reported sequence were also observed at several positions: –583 bp (T insertion), –507 bp (C insertion), –71 bp (T deletion), +17 bp (C insertion), and +121 bp (GC insertion).

It is well established that the occurrence of KD may be six times more likely in Korean children than in Caucasian.[24] We suggested that these interethnic variations in the frequencies of polymorphisms might be associated with different frequencies of KD occurrence. Potentially, M1 and M2 polymorphisms of IL-6 promoter gene in Korean children may contribute to frequent incidence of KD.

By preparing IL-6 promoter-luciferase fusion constructs (pGL3-IL-6-wild type, –M1, –M2, –(M1+M2)) and transient transfection assays in CV-1 and HeLa cells, we tested the functional significances of the polymorphisms (M1 and M2) and showed different results in each cell type. The reasons for such difference in the CV-1 and HeLa cells may be attributed to the different susceptibility to IL-1 and LPS in different cell types. Especially the inflammatory cytokines such as IL-6 may be more important in the organs prone to infection, such as CV-1 cells from kidney rather than HeLa cells from cervix.

In CV-1 cells, mutations at these positions significantly

decreased luciferase expression. Repressive activity of M1 is less potent than that of M2. Repressive activities of M1 and M2 have additive activity. However, these two mutations significantly increased luciferase expression with the stimulation of IL-1 and LPS. After stimulation with IL-1 and LPS, M2 shows more increased luciferase expression than M1. And also M1 and M2 mutations have additive activity of increased luciferase expression. LPS has more potent stimulatory activity than IL-1, in contrast to Fishman report[20] in which IL-1 had more potent stimulatory activity. These results suggested that the promoter elements at which the mutations were introduced are important in transcriptional activation of IL-6 gene. IL-1 and LPS potently stimulated luciferase expression on the wild-type and mutant reporter constructs, suggesting that the mutations may affect the interaction with transcriptional factors critical in the transcription of IL-6. With these results, we may explain that an increased incidence of KD in Korean children is in part from these polymorphisms in IL-6 gene.

Computational search for transcription factors binding site of IL-6 gene promoter shows that GCF-CS, SV40 T-Ag and Sp-1 can bind to the wild-type M1 region (+162 bp, +168 bp). When the region is mutated into M1, three more transcription factors (AP-2, CTCF-RS, APRT-mouse) can potentially bind to the region. At the wild-type M2 region (–594 bp), no transcription factor binds to the region, but once mutated, SV40 T-Ag, CTRF-RS and PuF-RS can potentially recognize the M2 mutated sequence. These alterations in transcription factor binding may be important in the transcriptional regulation of IL-6 gene. However, we do not know interactions among these transcription factors and which mechanisms are involved in the increase of IL-6 gene expression by IL-1 and LPS.

Materials and methods

Patients and controls

Blood samples were obtained from 10 patients (four females) with KD, who were admitted at the Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. All patients satisfied at least five of the six diagnostic criteria KD. The mean age was 2.9 years (range from 9 months to 5 years). All patients were treated with IVGG in addition to the high-dose aspirin therapy. Whole bloods were collected at the acute dignostic phase, before the initiation of any therapy. Ten normal children without any medical history of KD had their blood samples taken before undergoing elective surgical procedures. The mean age was 3.1 years (range from 1 to 5 years). The informed consents were obtained from the parents of the children included in this study.

Extraction of genomic DNA

Genomic DNA was extracted from the whole blood samples of the patients and the controls using E.Z.N.A. Blood DNA kit (Omega Bioteck, GA, USA).

PCR

The sequences of primers are given in Table 1. Two enzyme sites, *Kpn*I and *Hind*III, were incorporated into forward and reverse amplified primers used to amplify the promoter region of IL-6 gene. These primers amplified promoter of 858 bp, starting upstream of the distal putative glucocorticoid response element and finishing adjacent to the main translation start site. The cycling conditions were: denaturation at 94°C for 3 min, 35 cycles of amplification (94°C 30 sec, 66°C 1 min, and 72°C 1 min) and final 72°C, 10 min. PCR products were analyzed by 2% agarose gel electrophoresis.

Table 1 Sequences of used primers

Primers used to generate PCR products for analysis of IL-6 gene	
DF20	5′-GGAGTCACACACTCCACCT-3′
DF21	5′-GTGACTGACAGCACAGCT-3′
IL6 *Kpn*I	5′-GATCGGTACCGGAGTCACACACTCCACCTGGAGACGCC-3′
IL6 *Hind*III	5′-GCTAGAAGCTTGTGACTGACAGCACAGCTGGGAGCCTGC-3′
Primers used for manual sequencing	
MIS	5′-AGAGTAAAGCTGAAGTCATGCACGAAG-3′
MIR	5′-CTTCGTGCATGACTTCAGCTTTACTCT-3′
MS	5′-ATATTTATTGGGGGTTGAGACTCTAAT-3′
MR	5′-CAAATGTGGGATTTTCCCATGAGTCTCAA-3′
Primers used for PCR-based site-directed mutagenesis	
Mut 810 Forward	5′-GAGGGGTGTGTGGCCCAGGGATGCGGGGCGCCAGCAGAGGCAGGCTC-3′
Mut 810 Reverse	5′-GAGCCTGCCTCTGCTGCTGGCGCCCCGCATCCCTGGGCCACACACCCCTC-3′
Mut 60 Forward	5′-GCACGAAATTTGAGGATGGCCAGGCAGTCTAC-3′
Mut 60 Reverse	5′-GTAGACTGCCTGGCCATCCTCAAATTTCGTGC-3′
Primers used for confirming site-directed mutagenesis	
T7 promoter within pT7	5′-TCTAATACGACTCACTATAGG-3′
U-19 mer within pT7	5′-GGTTTTCCCAGTACGCGACG-3′

Sequencing
The PCR products were purified with QIAqick gel extraction kit (Qiagen, CA, USA) and sequenced with automatic DNA sequencer (ALF express, Amersham Pharmacia, Uppsala, Sweden) using primer sets shown in Table 1.

Cloning of PCR products
PCR products were seperated by 2% agarose gel electrophoresis and purified using Quiagen II gel extraction kit (Quiagen). Purified PCR products were digested with *Kpn*I and *Hind*III and cloned into pT7-blue script KS/*Kpn*I·*Hind*III vector (Novagen, WI, USA). Then recombinant plasmid DNA was purified using QIAprep miniprep kit (Qiagen). Promoter sequences were determined by automatic DNA sequencer (ALF express).

Site-directed mutagenesis
Using pGL3-IL-6 promoter-luciferase as template, PCR-based site-directed mutagenesis was performed using Quick Change Site-Directed Mutagenesis Kit (Stratagene, CA, USA) (Table 1). The cycling conditions were: 95°C for 30 sec, then 18 cycles of 95°C for 30 sec, 55°C for 1 min, and 68°C for 7 min. DNA was further treated with *Dpn*I at 37°C for 1 h. Transformation into Epicurian Coil XL1-Blue Supercompetent cells was performed with 1 μl of digested DNA. After mini-scale preparation of plasmids, mutation was confirmed by DNA sequencing using T7 sequences primer (Table 1).

Il-6 promoter-luciferase reporter fusion plasmid constructs
Plasmid containing mutation was digested with *Hind*III, then filled-in with Klenow and dNTP, and further digested with *BamH*I. Then IL-6 promoter restructing fragment/Blunt·*BamH*I was cloned to pGL3 Basic Luciferase vector/*Sam*I·*Bgl*II.

Transient transfections
Five × 10^5 monkey kidney cell line CV-1 cells and human cervix cell line HeLa cells were seeded into each well of a six-well plate and grown to confluence in Dulbecco's modified Eagle's medium (GIBCO BRL, MD, USA) supplemented with 10% FCS, non-essential amino acids and penicillin 50 U per ml/streptomycin 50 μg per ml (Sigma, MO, USA). CV-1 and HeLa cells were transfected with 0.6 μg of reporter plasmid and 50 ng of pCMV-β-gal which lacks a cytokine responsive promoter using Lipofectamin plus transfection reagent (GIBCO BRL). After 24 h, the cells were stimulated with either 10 U/ml of IL-1 (R & D Systems, MN, USA) or 10 μg/ml of LPS (Sigma). After incubating the cells another 24 h, the cells were harvested. Luciferase and β-galacosidase expression were measured using commercial assay kits (Promega, WI, USA). Transfection was repeated three times. Transient efficiencies and cell lysate recovery were normalized with β-galactosidase activities.

Measurement of serum IL-6
The serum samples were obtained from KD patients in the acute phase at the time of diagnosis, before the initiation of any therapy. Serial samples were obtained from the patients subsequently in the subacute phase, when the patients were afebrile. The samples were stored at –70°C until measured. IL-6 concentration was measured in duplicate using a commercially available ELISA kit (R&D Systems).

Statistical methods
The concentrations of serum IL-6 between acute and subacute phase of disease were compared by utilizing paired *t*-test. Analysis of variance was used in comparing levels of luciferase among the constructs. Statistical significance was considered to be at 0.05 levels.

References

1 Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children: Clinical observations of 50 cases. *Jpn J Allerg* 1967; **16**: 178–222.
2 Kawasaki T, Kosaki F, Osawa S, Shigematsu I, Yanagawa S. A new infantile acute febrile mucocutaneous lymphnode syndrome (MCLS) prevailing in Japan. *Pediatrics* 1974; **54**: 271–276.
3 Leung DYM, Chu ET, Wood N, Grady S, Meade R, Gdha R. Immunoregulatory T cell abnormalities in MCLS. *J Immunol* 1983; **130**: 2002–2004.
4 Furukawa S, Matsubara T, Jujho K *et al*. Peripheral blood monocyte macrophage and serum tumor necrosis factor in Kawasaki disease. *Clin Immunol Immunopathol* 1988; **48**: 247–251.
5 Furukawa S, Matsubara T, Motohashi T, Nakachi S, Sasai K, Yabuta K. Expression of FcεR2/CD23 on peripheral blood macrophages/monocytes in Kawasaki disease. *Clin Immunol Immunopathol* 1990; **56**: 280–286.
6 Laxer RL, Schffer FL, Myones B *et al*. Lymphocyte abnormalities and complement activation in Kawasaki disease. *Prog Clin Biol Res* 1987; **250**: 175–184.
7 Kim DS, Han BH, Lee SK, Lee HK, Chaw YJ, Lee KY. Evidence for selection of 11 amino acid CDR3 domains in VκIII-derived immunoglobulin light chains in Kawasaki disease. *Scand J Rheumatol* 1997; **26**: 350–354.
8 Kim DS, Hwang HY, Yu CJ. Oligoclonal expansion of the VH family in Kawasaki disease. *J Korean Pediatr Soc* 1999; **42**: 1246–1254.
9 Lang BA, Silveramn ED, Laxer RM, Lau AS. Spontaneous tumor necrosis factor production in Kawasaki disease. *J Pediatr* 1989; **115**: 939–943.
10 Maury CPJ, Salo E, Pelkonen D. Circulating interleukin-1β in patients with Kawasaki disease. *N Engl J Med* 1988; **319**: 1670–1671.
11 Kim DS. Serum interleukin-6 in Kawasaki disease. *Yonsei Med J* 1992; **33**: 183–188.
12 Ueno Y, Takano N, Kanegane H *et al*. The acute phase nature of interleukin-6: studies in Kawasaki disease and other febrile illness. *Clin Exp Immunol* 1989; **76**: 337–342.
13 Hirano T, Akira S, Taga T, Kishimoto T. Biological and clinical aspects of interleukin-6. *Immunol Today* 1990; **11**: 443–449.
14 Kim MK, Kim DS. The interleukin-6 level in Kawasaki disease. *J Korean Pediatr Soc* 1992; **35**: 515–525.
15 Olomolaiye OO, Wood NAP, Bidwell JL. A novel NlaIII polymorphism in the human IL-6 promoter. *Eur J Immunogen* 1998; **25**: 267–268.
16 Faulds G, Fishman D, Woo P, Humphries S. Novel FokI polymorphism in the 5′ flanking region of the human IL-6 gene. Genbank/EMBL, Accession number AF048692.
17 Nakajima T, Ota N, Yoshida H, Watanabe S, Suzuki T, Emi M. Allelic variants in the interleukin-6 gene and essential hypertension in Japanese women. *Genes Immun* 1999; **1**: 115–119.
18 Osiri M, McNicholl J, Moreland LW, Bridges SL Jr. A novel single nucleotide polymorphism and five probable haplotypes in the 5′ flanking region of the IL-6 gene in African-Americans. *Genes Immun* 1999; **1**: 166–167.
19 Jordanides N, Eskdale J, Stuart R, Gallagher G. Allele associations reveal four prominent haplotypes at the human interleukin-6 (IL-6) locus. *Genes Immun* 2000; **1**: 451–455.

20 Linker-Israeli M, Wallace DJ, Prehn JL, Nand R, Li L, Klinenberg JR. A great variability in the 3′ flanking region of the IL-6 gene in patients with systemic lupus erythematosus (SLE). *Autoimmunity* 1996; **23**: 199–209.
21 Papassotiropoulos A, Bagli M, Jessen F *et al*. A genetic variation of the inflammatory cytokine interleukin-6 delays the initial onset and reduces the risk for sporadic Alzheimer's disease. *Ann Neurol* 1999; **45**: 666–668.
22 Fishman D, Faulds G, Jeffery R *et al*. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998; **102**: 1369–1376.
23 Collado-Escobar MD, Nieto A, Mataran L, Raya E, Martin J. Interleukin 6 gene polymorphism in not associated with ankylosing spondylitis. *J Rheumatol* 2000; **27**: 1461–1463.
24 Mason WH, Takahashi M. Kawasaki syndrome. *Clin Infect Dis* 1999; **28**: 169–187.