

# Production of Plasma Leptin and Expression of Interferon- $\gamma$ Inducible Protein-10 (IP-10), Monokine Induced by Interferon- $\gamma$ (Mig) and Interleukin-8 (IL-8) mRNA in Kawasaki Disease

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## ABSTRACT

**Background:** Kawasaki disease is an acute febrile illness with systemic vasculitis which primarily affects children. We examined the production of leptin in plasma and gene expressions of CXC chemokines in peripheral blood mononuclear cells from patients with Kawasaki disease. **Methods:** Consecutive 39 samples from 13 patients according to the different clinical stages (acute, subacute, convalescent) of Kawasaki disease were collected. The plasma leptin levels according to clinical stages of Kawasaki disease were examined by ELISA and the expression of IP-10, Mig and IL-8 mRNAs in 39 samples (13 samples of each stage) from 13 cases were examined by RT-PCR. **Results:** There were not significant changes of plasma leptin levels according to the clinical stages of Kawasaki disease. The mean values of plasma leptin concentrations during each of the stages ( $n=13$ ,  $p>0.05$ , pg/ml) were  $335.8\pm549.0$  in acute,  $358\pm347.6$  in subacute, and  $443.6\pm645.9$  in convalescent stage. The mRNAs of IP-10, Mig, and IL-8 were expressed in 13/13 (100%), 2/13 (15%), 9/13 (69%) during acute stage, 13/13 (100%), 6/13 (46%), 13/13 (100%) during subacute stage, and 13/13 (100%), 4/13 (31%), 10/13 (77%) during the convalescent stage, respectively. In three patients, the production of leptin and expression of IP-10 mRNA were dramatically decreased according to the process of the clinical stages. In five patients with prominent cervical lymphadenopathy, the expression of IL-8 mRNA during the subacute stage was more elevated than the acute and convalescent stages. **Conclusion:** This data suggests that the production of leptin and the gene expressions of IP-10, Mig and IL-8 seem to have no significant correlation to the clinical stages of Kawasaki disease. However, expression patterns of IP-10, Mig and IL-8 mRNA may be related to the specific clinical manifestations, and the expression of IP-10 may also be correlated to leptin levels with pericardial involvement. (*Immune Network* 2002;2(4):202-207)

**Key Words:** Leptin, IP-10, Mig, IL-8, Kawasaki disease

## Introduction

Kawasaki disease, first described by Kawasaki in 1967, is an acute systemic vasculitis occurring in early childhood (1). Although the clinical and epidemiological features suggest an infectious origin, its etiology remains unknown. The interaction between leukocytes and vascular cell walls contributes to the

pathogenesis of vasculitis, and it is important to understand the factors that recruit and activate leukocytes to the region of vasculitis in Kawasaki disease. Many studies (2-5) suggest that cytokines play an important role in the onset of this disease. Recently, several studies (6-10) have been concerned about chemokines, a large family of structurally related small proteins of 8~10 kDa sharing the ability to induce chemotaxis and tissue extravasation and to modulate various functions of leukocytes. Chemokines (11) are important for the recruitment of leukocytes to sites of infection, which is essential in host defense and may lead to clearance of inciting factors, and have been detected in body fluids and tissues in

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a variety of pathologic conditions. However, chemokine interferon- $\gamma$ (IFN- $\gamma$ )-inducible protein-10 (IP-10) and monokine induced by IFN- $\gamma$  (Mig) have not been investigated in this disease. In addition, there has not been any data produced about the relationship of plasma leptin to the expression of these chemokines and clinical stages in Kawasaki disease.

Leptin is a 16-kDa nonglycosylated peptide hormone encoded by the *ob* gene and synthesized exclusively in adipocyte (12). It appears to be important in the regulation of glucose metabolism, insulin secretion and body weight (13). The most well-known function of leptin is considered to regulate food intake. However, leptin is a pleiotropic molecule which affects cytokine production, the activation of monocytes/macrophages, wound healing, angiogenesis and hematopoiesis (14). Leptin production is increased acutely during infection and inflammation, and leptin can also directly activate the inflammatory response (15).

In the present study, we measured plasma levels of leptin and evaluated the expression of IP-10, Mig and IL-8 genes in peripheral blood mononuclear cells according to the clinical stages of Kawasaki disease.

## Patients and Methods

**Patients and controls.** The study was composed of 13 consecutive patients (Table I) who met the diagnostic guidelines for Kawasaki disease as described elsewhere (1). All patients received a high dose single intravenous gamma globulin (IVIG) infusion (Livgamma<sup>®</sup> 2 gm/kg in 10 to 12 hours) in combination with oral aspirin (Rhonal<sup>®</sup> 60~100 mg/kg divided

prospectively evaluated at Yeungnam University Hospital, Daegu, Korea. All patients underwent serial blood sampling and echocardiographic evaluation. The duration of illness, at the time when the blood sampling and echocardiographic study was done, was as follows: acute stage - before treatment with IVIG and aspirin, subacute stage - 7 to 10 days after intravenous gamma globulin treatment, and convalescent stage - 30 to 40 days after onset of the Kawasaki disease. None of the patients died. Informed consent was obtained from the parents of the patients included in this study.

**Plasma, PBMCs and total RNA isolation.** plasma and peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation on a Ficoll-Hypaque gradient at 2,000 rpm for 30 min, then the total RNA was isolated with the use of Trizol solution as instructed by the manufacturer. Briefly, after the addition of 1 ml of Trizol and 200 $\mu$ L of chloroform followed by centrifugation, the aqueous phase was combined with an equal volume of isopropanol. The precipitated pellet was washed with 70% ethanol and suspended again in diethylpyrocarbonate (DEPC)-treated water. Plasma samples were frozen at -70°C until radioimmunoassay.

**RIA for plasma leptin.** RIA for the concentration of leptin was performed according to the manufacturer's instructions from Linco Systems (St. Charles, MO, USA). Briefly, 100 $\mu$ L of assay buffer was added to 100 $\mu$ L of each sample in duplicate. Then, each 100  $\mu$ L of <sup>125</sup>I-Human Leptin and 100 $\mu$ L of Human Leptin antibody was added. The mixture was incu-

**Table I.** Baseline characteristics and the concentrations of plasma leptin in patients with Kawasaki disease

Patients	Sex	Age (Mo)	W (kg)	H (cm)	BMI	Specific findings
P1	M	28	13	92	15.3	
P2	M	10	10.8	73.8	20*	PE, CL
P3	F	23	11.5	86	15.5	PE
P4	M	18	11.1	85	15.4	
P5	F	17	12	82	17.9	CL
P6	F	10	7.8	69	16.3	CAD
P7	F	12	9.2	76.3	15.9	
P8	M	36	16	93	18.6*	Meningitis, CL
P9	F	36	15	96.9	16	CL
P10	F	12	9.6	78	15.7	CAD
P11	F	24	15	98	15.6	
P12	M	24	17.5	99.9	17.5	LV, CL
P13	M	24	17.5	89	22.2*	CAD

W: weight, H: height, BMI: body mass index, PE: pericardial effusion, CL: cervical lymphadenopathy, CAD: coronary artery dilatation, LV: decreased function of left ventricle.

\*: obesity, >95% in BMI percentile chart in children.

into 3 equal doses) treatment in acute phase, and were

bated for 24 hours at 4°C. Then, 1 mL of cold pre-

cipitating reagent was added and the mixture was further incubated for 20 minutes at 4°C. Bound and free ligands were separated by centrifugation. Leptin levels were counted in a gamma counter (Cobra II, Packard) for 1 minute.

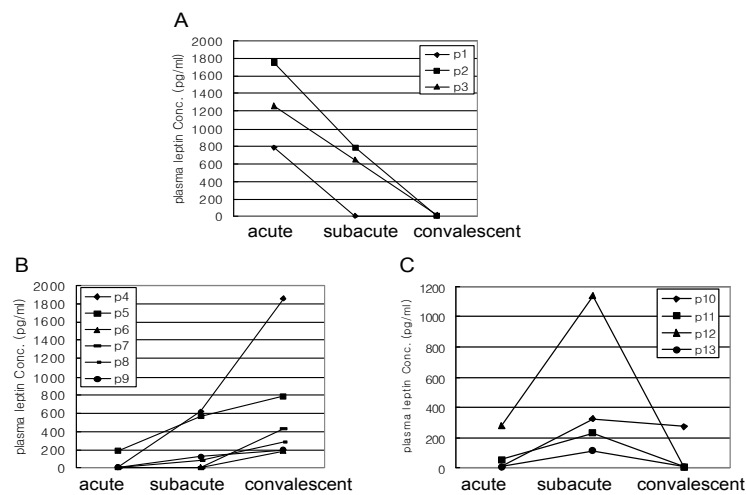
*Reverse transcriptase-polymerase chain reaction (RT-PCR).* One µg of total RNA per sample was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Perkin Elmer, Norwalk, CT, USA) and oligo dT priming according to the manufacturer's instruction, at 42°C for 15 minutes. Amplification with specific primers was performed in a Gene Amp PCR system 9600 (Perkin Elmer) for 31 cycles with a 45 s/94°C denaturation, 1 min/59°C annealing, 1 min/72°C extension profile in the case of IP-10; for 35 cycles with a 45 s/94°C denaturation, 45 s/61°C annealing, 45 s/72°C extension profile in the case of Mig; for 35 cycles with a 15 s/95°C denaturation, 30 s/60°C annealing, 1 min/72°C extension profile in the case of IL-8; for 40 cycles with a 1 min/94°C denaturation, 1 min/55°C annealing, 1.5 min/72°C extension profile in the case of TNF-α; for 30 cycles with a 30 s/95°C denaturation, 30 s/60°C annealing, 30 s/72°C extension profile in the case of GAPDH. Amplification of mRNA for the housekeeping gene

Amplified products were electrophoresed on 1.5~2% agarose gel stained with 0.5 g/mL ethidium bromide. The primer sequences were as follows: GAPDH (178 bp) sense; 5'-accactctccacctttg-3', antisense; 5'-ctcttgctcttctgctggg-3', IL-8 (300 bp) sense; 5'-atgacttccaagctggccgtg-3', antisense; 5'-ttatgaattctcagcccttcaaaaacttctc-3', IP-10 (107 bp) sense; 5'-ggaacc tccagtctcagcacc-3', antisense; 5'-gcgtacggttctagagagaggtac-3', Mig (123 bp) sense; 5'-ttctcttgg gcatcatcttgctg-3', antisense; 5'-ggctttcaaggattgtaggtgga-3'.

*Statistical analysis.* Results were presented as mean ± SD. Statistical differences were analyzed by the paired t-test. Values were considered significant when p < 0.05.

**Results**

*Plasma levels of leptin in Kawasaki disease.* We first examined the plasma leptin levels according to clinical stages of Kawasaki disease. The production of plasma leptin according to clinical stages showed 3 patterns, namely, descending (Fig. 1A), ascending (Fig. 1B), and reverse V shape (Fig. 1C). In descending pattern, two of three cases had pericardial effusion. Three of nine cases which showed ascending pattern had severe cervical lymphadenopathy, and two of four



**Figure 1.** Three patterns of plasma leptin levels in patients with Kawasaki disease. Serial values before (acute), at 1~3 days (subacute) and at 2~3 weeks (convalescent) after the gamma globuline infusion are shown. A: acute, B: subacute, C: convalescent. GAPDH was used as an internal quality standard.

cases which showed reverse V shape pattern had

coronary artery dilatation. However, as described in specific findings in Table I, there were not significant differences in patterns of leptin levels between specific clinical findings. To gain a further insight into plasma leptin levels, the expression of leptin receptor, long form (OB-R<sub>L</sub>) from the PBMC in three cases which showed the descending pattern of plasma leptin (Fig. 1A) was examined. The expressions of OB-R<sub>L</sub> were gradually increased according to the clinical stages. The mean values of plasma leptin concentrations during each of the stages (n=13, *p* > 0.05, mean±SD, pg/ml) were 335.8±549.0 in acute, 358±347.6 in subacute, and 443.6±645.9 in convalescent stage (Table II). The gender, age, and body mass index (BMI) in all cases were not related to the patterns of leptin levels during the clinical stages. Consequently, there was a negative correlation between the plasma leptin and clinical stages in Kawasaki disease.

*Expression of chemokine IP-10, Mig, and IL-8 in Kawasaki disease.* The expression of IP-10, Mig and IL-8 mRNAs in 39 samples (13 samples of each stage) from 13 cases were examined. Expressions of IP-10, Mig, IL-8 mRNA were detected in 13/13 (100%), 2/13 (15%), 9/13 (69%) during the acute stage, 13/13 (100%), 6/13 (46%), 13/13 (100%) during the subacute stage, and 13/13 (100%), 4/13 (31%), 10/13 (77%) during the convalescent stage, respectively (Table III). The lowest rate of expression was shown in Mig mRNA. We could not find any relationship between the clinical manifestations in patients and the

**Table II.** Mean values of plasma leptin

Acute	Subacute	Convalescent
335.8±549.0*	358.3±347.6 <sup>†</sup>	443.6±645.9 <sup>‡</sup>

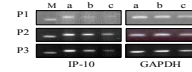
n=13, mean±standard deviation (pg/mL)  
 \**p*=0.137, compared with subacute, <sup>†</sup>*p*=0.754, compared with convalescent, <sup>‡</sup>*p*=0.212, compared with acute

**Table III.** Summary of expression of chemokine genes in Kawasaki disease

	Acute	Subacute	Convalescent
IP-10	13/13 (100)	13/13 (100)	13/13 (100)
Mig	2/13 (15)	6/13 (46)	4/13 (31)
IL-8	9/13 (69)	13/13 (100)	10/13 (77)

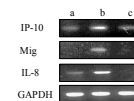
Values given in parentheses are the percent of positive patient samples.

expression patterns of Mig mRNA during the clinical



**Figure 2.** The expression profiles of IP-10 mRNA during the 3 phases in three patients with Kawasaki disease. P2 and P3 accompanied pericardial effusion. Total RNA was isolated from PBMC collected from Kawasaki patients, and RT-PCR was performed with primers indicated as described in materials and methods. GAPDH was used as an internal standard. M: DNA marker (100 bp DNA ladder) a: acute, b: subacute, c: convalescent.

stages. The expression of IP-10 mRNA was detectable in all samples over the three clinical stages. In the group of descending pattern of plasma leptin levels, the expression of IP-10 mRNA was gradually decreased according to the clinical stages (Fig. 2). Pericardial effusion was the distinguished clinical finding in two of three cases. In all 5 cases with massive cervical lymphadenopathy (lymph node size >5 cm), the expression of IL-8 mRNA during the subacute stage was more elevated than acute and convalescent stages (Fig. 3). The production pattern



**Figure 4.** Expression pattern of the genes for chemokines in a Kawasaki patient (P8) with meningitis. Total RNA was isolated from PBMC collected from Kawasaki patients, and RT-PCR was performed with primers indicated as described in materials and methods. GAPDH was used as an internal standard. a: acute, b: subacute, c: convalescent.

of plasma leptin in each of these cases was different,

as shown in Fig. 1. We could also find a specific pattern in the expressions of IP-10, Mig, IL-8 mRNA in one case with meningitis. The expressions of all cytokine mRNAs during the subacute stage were more elevated than other stages (Fig. 4). In this case, the production of plasma leptin during the clinical stages showed an ascending pattern (Fig. 1B).

## Discussion

In the present study, we analyzed the production of plasma leptin and the expression of CXC chemokine IP-10, Mig, and IL-8 mRNA according to the clinical stages in Kawasaki disease. Earlier studies (5-8,10) on the production or expression of chemokines in Kawasaki disease have focused mainly on CC chemokines or IL-8. Moreover, a leptin study of Kawasaki disease has not been performed yet.

Although we found three patterns of plasma leptin kinetics according to clinical stages, there was no specific correlation between leptin levels and the clinical stages of Kawasaki disease. Increased serum leptin levels have been found in patients with alcoholic cirrhosis (16) or during pregnancy (17). In sepsis, either increases or no changes in leptin levels have been reported (18-20). However, no correlation between leptin levels and disease activity has been found in rheumatoid arthritis (21) and inflammatory bowel disease (22).

Kawasaki disease is associated with the activation of peripheral blood macrophages/monocytes, T cells, B cells, and clonal expansion of T cells, resulting in highly elevated levels of various cytokines (23-25). Pathophysiologic roles of chemokines in Kawasaki disease are still poorly understood. CXC chemokines are mostly chemotactic for neutrophils, but CXC chemokine IP-10 is a chemoattractant for monocytes and T cells, and Mig is chemotactic for tumor infiltrating lymphocytes and activated T cells. Wong et al (6) have reported that there was no obvious correlation between clinical stages of the Kawasaki disease and expression of chemokine RANTES, MCP-1 and MIP-1 $\beta$ . Our data with IP-10, Mig, and IL-8 also showed no obvious correlation between clinical stages. However, we observed that some expression patterns of IP-10 and IL-8 might be related to the specific clinical manifestations of Kawasaki disease. In three patients especially, the expression pattern of IP-10 according to the clinical stages coincided with the pattern of plasma leptin production. Namely, both IP-10 expression and leptin production gradually decreased according to the process of clinical stages. However, we could not find any specific findings in common among three patients except pericardial effusion in two of three cases.

IL-8 has been suggested as an indicator of high

risk for coronary lesions in Kawasaki disease (5). In our data, the expression of IL-8 mRNA in all 5 cases with severe cervical lymphadenopathy was over-expressed in subacute stages, compared with other stages. There have been various studies of IL-8 modulation with IVIG in Kawasaki diseases (8,10,26). In a study by Asano et al (26), the expression of IL-8 was shown to increase after IVIG, and Terai et al (7) have reported that IVIG treatment correlated with a rapid decrease in the circulating levels of MCP-1 but not IL-8. In an *In vitro* study with human monocyte (27), IVIG has also been shown to enhance the expression of IL-8 mRNA and protein production. All samples of the subacute stage in this study were obtained from the patients after IVIG therapy. However, the IL-8 mRNA in other samples except 5 cases was not over-expressed during subacute stage, and in two cases with coronary artery dilatation, the IL-8 mRNA was expressed evenly during the three clinical stages. Therefore, further discrete study of the kinetics of IL-8 in Kawasaki disease must be performed.

In a patient with meningitis, the expressions of IP-10, Mig, and IL-8 mRNA during subacute stage were more elevated than during other stages. Chemokine production has been demonstrated in the cerebrospinal fluid in infectious meningitis (28), and there is some suggestion that IL-8, MCP-1 and IP-10 are probably the most important chemokines in the pathophysiology of meningitis (29). But, we could not find any reports for a cytokine network between Kawasaki disease and meningitis. To confirm our data about Kawasaki disease with meningitis, study with more cases must be performed.

Although, the number of cases in this study was not sufficient, these results suggest that plasma leptin does not play a role as a factor participating in pathophysiologic functions in Kawasaki disease, however, the action mechanism of IVIG and additional specific clinical manifestations may contribute to the expression patterns of chemokine IL-8 and IP-10, Mig in Kawasaki disease.

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