

Serum Interleukin-6 in Kawasaki Disease

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Kawasaki disease (KD) is an acute febrile illness of infancy and early childhood. In spite of extensive studies, the cause of KD is not known. Interleukin 6 (IL-6) has manifold biological functions involved in the immune or inflammatory responses of the host to various stimuli. Here the author investigated whether IL-6 might be responsible for manifestations of KD, such as immunoglobulin hypersecretion, lymphocyte activation and systemic vasculitis. Serum IL-6 levels in KD were determined by ELISA. Usually sera from healthy children contained only negligible levels of IL-6. Serum IL-6 was markedly elevated in all patients with acute KD, which gradually decreased during the course of the disease. Serum IL-6 correlated with serum concentration of C-reactive protein and with serum soluble interleukin-2 receptor level, but did not show any correlation with peak platelet count during subacute phase of the disease. Increased serum IL-6 level did not show any relation to development of coronary aneurysms and dilatation. Further studies will be needed to examine the source and the pathogenetic roles of increased serum IL-6 in KD.

Key Words: Kawasaki disease, interleukin 6, soluble interleukin-2 receptor, c-reactive protein

Kawasaki disease is an acute febrile illness that primarily affects infants and young children. It was first described in Japan in 1967 by Dr. Tomisaku Kawasaki (Kawasaki 1967). Although KD is clearly most prevalent in Japan, KD has been reported throughout the world in children of all races including Korean children. The histologic feature of KD in the acute febrile stage is characterized by vasculitis of small vessels, perivasculitis of large arteries and acute pancarditis (Landing 1987). In spite of extensive studies, the cause of KD is not well known. However there has been much interest in the possibility that immunoregulatory abnormalities

play an important role in the pathogenesis of this disease. Among these abnormalities, increased numbers of activated B cells spontaneously secreting IgG and IgM have been found (Leung 1989). It is well known that interleukin-6 is a cytokine that stimulates terminal differentiation of the B cell to secrete immunoglobulins and it is also one of the cytokines that are abnormally increased in various autoimmune diseases including rheumatoid arthritis, and systemic lupus erythematosus (Hirano 1990). Interestingly, interleukin-6 is elevated in SLE with vasculitis but not in SLE without vasculitis (Kim et al 1991). As mentioned previously, KD is characterized by systemic vasculitis and increased numbers of activated B cells. So the increased serum interleukin-6 level in KD was studied.

MATERIAL AND METHOD

Patients

Thirty seven serum samples were obtained from 30 patients (18 male) with KD admitted at the Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. All patients fulfilled at least five

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Abbreviations: KD; Kawasaki disease, IVGG; Intravenous gamma globulin, IL-1; Interleukin 1, IL-6; Interleukin 6, TNF; Tumor necrosis factor, CRP; C reactive protein, SLE; Systemic lupus erythematosus, ALL; Acute lymphocytic leukemia, sIL-2R; Soluble interleukin 2 receptor, ELISA; Enzyme linked immunosorbent assay, IFN-gamma; Interferon gamma

of the six criteria for the diagnosis of KD. The mean age was 3 years (range 3 months to 11 years). All the patients received treatment with IVGG in addition to aspirin in high doses. Serial echocardiograms were obtained during the acute phase (day 3 to 9), the subacute phase (days 15 to 42: mean day 21), and the convalescent phase (over 6 weeks) of the illness to detect coronary aneurysms and dilatation. Twenty one serum samples were tested for serum interleukin-6 (IL-6) levels, C-reactive protein (CRP), and soluble interleukin-2 receptor (sIL-2R) levels at the time of diagnosis in the acute phase of the illness before any therapy. Subsequent sera obtained from patients during their illness included 16 samples obtained in the subacute phase, when patients were afebrile within 14 days of disease onset. Serial samples were obtained from 12 patients initially in the acute and subacute phase. Sera were obtained from 13 age-matched children with meningitis from enterovirus and 20 age-matched children who were having routine blood work before elective surgical procedures.

Serum interleukin-6 assay

Serum was stored at -70°C until measured for serum IL-6, which was measured by an ELISA kit, which was kindly supplied from Fujirebio Inc. (Tokyo, Japan).

Serum C-reactive protein assay

Serum was tested for CRP which was measured by fluorescence polarization immunoassay using TDx (Abbott Inc, North Chicago, IL)

Soluble Interleukin-2 receptor assay

sIL-2R assay was done by ELISA Kit (T Cell Sciences, Boston, MA)

Statistical analysis

The Student t test was used to detect differences between patients and control subjects. A paired t test was used to compare paired acute and subacute phase serum samples. Pearson simple correlation coefficients were calculated between interleukin 6 and other variables including C-reactive protein, soluble interleukin 2 receptor, and peak platelet count.

RESULTS

In all 20 healthy children, IL-6 levels in the serum were below 5 pg/ml. In contrast to the healthy

children, it should be noted that serum levels of IL-6 were markedly elevated in all patients with acute KD. Their concentrations ranged from 18.2 to 233.5 pg/ml: the mean was 123.0 pg/ml (Fig. 1). As shown in the Fig. 1, IL-6 levels in sera collected during the subacute phase of KD was significantly reduced compared with that during the acute phase of KD which ranged from undetectable to 57.0 pg/ml: the mean was 21.7 pg/ml. In order to know whether elevated serum levels of IL-6 might be seen exclusively in patients with acute KD, sera from patients with febrile disease, enteroviral meningitis were obtained and tested. Sera from patients with this disease also showed elevated levels of IL-6 equivalent to that seen in subacute KD: the mean was 25.7 pg/ml (Fig. 1).

Figure 2 represents the result of serial levels of IL-6 seen in KD. With the exception of one patient, all the patients of KD showed a gradual decrease of serum IL-6 levels after temperature normalization.

It has been shown that IL-6 has the biological activity to mediate the induction of a number of liver-derived acute phase proteins, such as CRP, haptoglobin, alpha 1-acid glycoprotein and alpha 1-anti-trypsin in vivo as well as in vitro (Castell et al. 1988; Geiger et al. 1988) Next, the correlation between serum IL-6 levels in KD and serum concen-

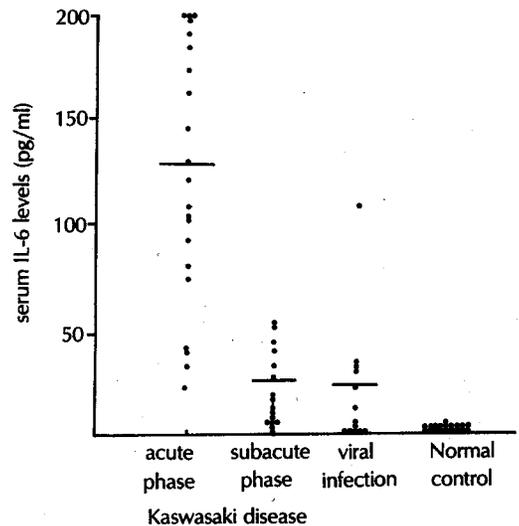


Fig. 1. Serum IL-6 levels in KD, viral meningitis, and normal children. The mean values are indicated by bars.

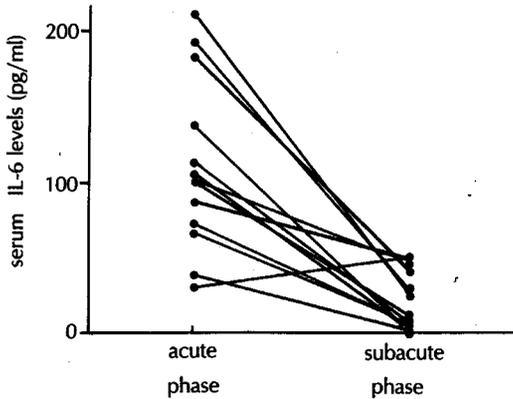


Fig. 2. IL-6 levels in serial serum samples of 13 patients with KD at the acute and subacute phase.

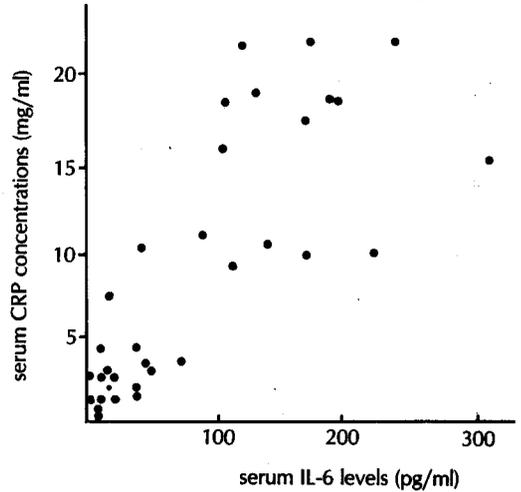


Fig. 3. Correlation between serum levels of IL-6 and CRP. ($r=0.73$ $p<0.001$)

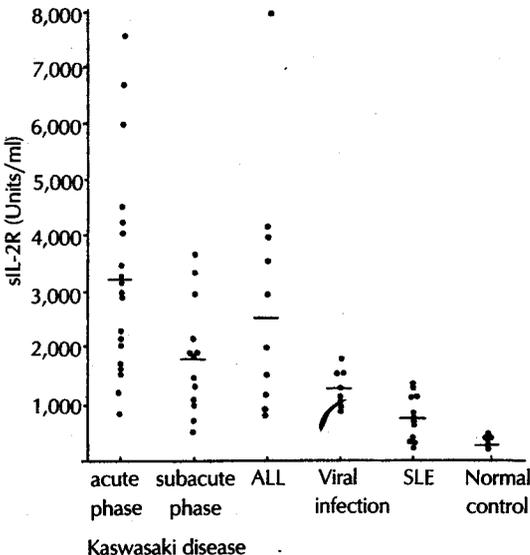


Fig. 4. Serum levels of sIL-2R in patients with Kawasaki disease, acute lymphocytic leukemia (ALL), enteroviral infection, systemic lupus erythematosus (SLE), and normal children.

trations of CRP was investigated. As shown in Figure 3, the serum levels of IL-6 and CRP were well correlated ($r=0.73$, $P<0.001$).

Looking for the evidence of immune activation in KD, as reflected by elevations in the serum sIL-2R levels, serum sIL-2R levels were tested. As shown in Figure 4, markedly elevated circulation levels of sIL-2R were found in patients' sera during the acute phase of KD. The acute phase sIL-2R level ranged from 809 to 7,569 U/ml: the mean was 3,275 U/ml. Serum sIL-2R levels remained significantly elevated in the subacute phase (mean 1,841 U/ml) compared with normal children (mean 315 U/ml), even though this group of patients were afebrile when tested (Fig. 4). Serum sIL-2R levels were also tested in patients with ALL, SLE, and virus infection. As shown in Figure 4, serum concentration of sIL-2R in ALL, SLE, and viral infection were elevated compared with normal controls (mean 2,379, 820, 1,290 respectively).

Paired samples from patients with KD were studied during the acute phase and subacute phase of their illness. As shown in Fig. 5, follow-up sIL-2R levels in these patients (1,841 U/ml) were significantly lower levels measured during their acute disease (3,275 U/ml).

Although IL-6 can be produced from various

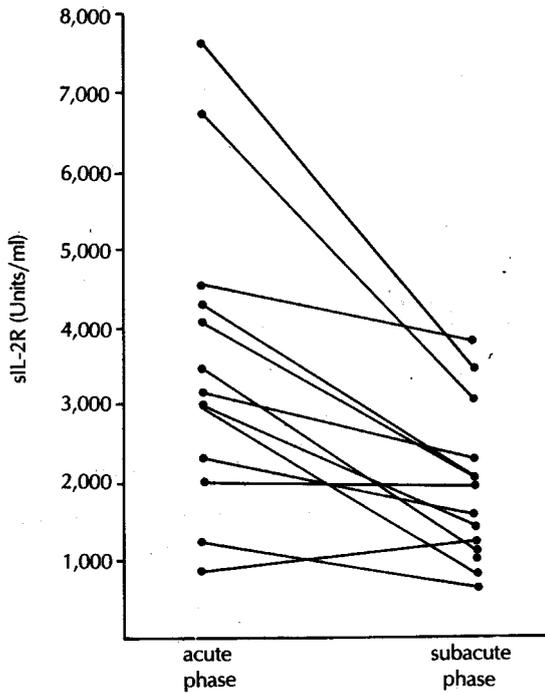


Fig. 5. Levels of sIL-2R in serial serum samples obtained from 13 patients during the acute phase of Kawasaki disease and subsequently during the subacute or convalescent phase of their illness.

kinds of immune cells, source of increased IL-6 levels of KD is not known well. Activated T cells can be speculated as a source of increased IL-6 levels in KD. The correlation of serum IL-6 levels and sIL-2R levels in KD was investigated. The serum levels of IL-6 levels and sIL-2R correlated well ($r=0.65$, $P<0.01$) (Fig. 6).

It has been shown that IL-6 has the biological

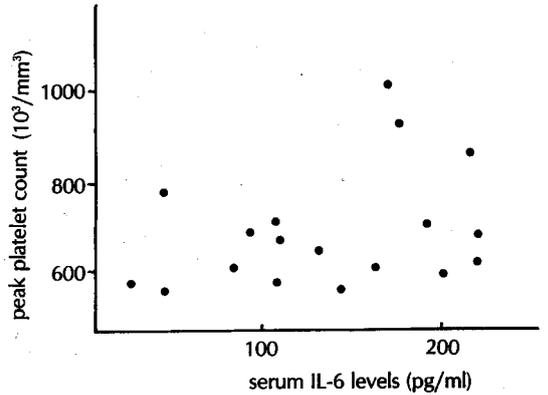


Fig. 7. Correlation between serum IL-6 levels at the acute phase of KD and peak platelet count at the subacute phase of disease. ($r=0.13$, $p>0.1$)

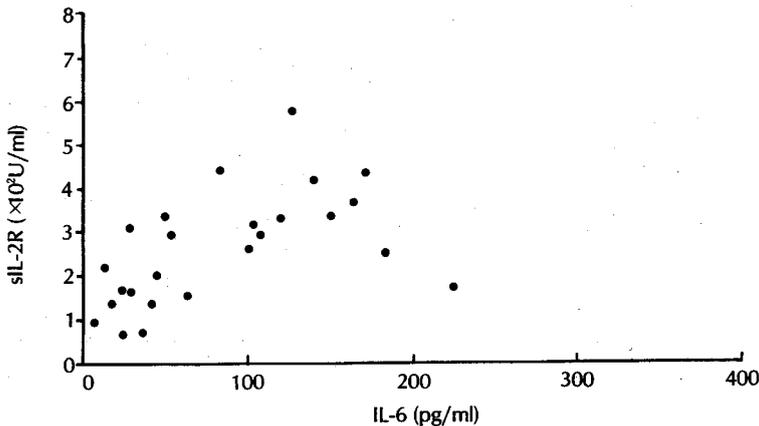


Fig. 6. Correlation between sIL-2R and IL-6. ($r=0.65$, $p<0.01$)

activity to stimulate the maturation of megakaryocytes. The correlation of serum levels of IL-6 during the acute phase of KD and peak platelet counts during the subacute phase of KD was studied. As shown in Fig. 7, there was no correlation between these two.

DISCUSSION

The underlying pathogenesis for the development of vasculitis in KD remains to be elucidated. The acute phase of KD is often accompanied by the activation of T cells and B cells (Leung 1989). The infiltration of macrophages and activated T cells has been observed in the vascular lesions of acute KD (Sugawara *et al.* 1987). In addition, the majority of patients with acute KD have been reported to have increased percentages of monocytes with spontaneous secretion of abnormally high levels of IL-1 (Leung *et al.* 1986), and an increase in serum TNF titer has been described in these patients (Furukawa *et al.* 1988). An increase TNF-alpha titer in the acute phase of these illness and a decrease TNF-alpha titer in the subacute phase of these illness (Data not shown) has also been observed. Leung *et al.* (1986) demonstrated that sera from the patients with KD contain IgM antibodies that can lyse cultured vascular endothelial cells stimulated with IFN-gamma. Furthermore, they have proposed that the cytolytic antibodies to IL-1 or IFN-gamma inducible endothelial cell surface antigens can contribute to the vascular injury observed during the acute phase of KD.

It is now known that IL-6 is a pleiotropic cytokine that is produced by a variety of cells and acts on a wide range of tissues, exerting growth-inducing, growth-inhibitory and differentiation-inducing effects, depending on the nature of the target cells. IL-6 is involved in the induction of B-cell differentiation, induction of acute phase proteins in liver cells, growth promotion of myeloma/plasmacytoma/hybridoma cells, inhibition of cell growth of certain myeloid leukemic cell lines and induction of their differentiation to macrophages, enhancement of IL-3-induced multipotential colony cell formation in hematopoietic stem cells and induction of the maturation of megakaryocytes as a thrombopoietic factor, induction of mesangial cell growth, induction of neural differentiation of PC12 cells, and induction of keratinocyte growth (Hirano *et al.* 1990). After abnormal production of IL-6 was first suggested to be related to polyclonal B-cell activation with

autoantibody production in patients with cardiac myxoma, IL-6 has been suggested to be involved in the pathogenesis of a variety of diseases, such as autoimmune diseases, plasma cell neoplasias and glomerulonephritis (Hirano *et al.* 1990).

On the basis of many biological activities of IL-6, in the present study the author's interests were directed to the involvement of IL-6 in the inflammatory responses and immunological disorders observed in patients of KD. Interestingly, serum IL-6 levels increased in patients of SLE with vasculitis but do not increase in patients of SLE without vasculitis (Kim *et al.* 1991). Here it can be speculated that increased IL-6 levels in patients with SLE had an important role of development of vasculitis in patients with SLE. KD is a disease of systemic vasculitis, which means that abnormal production of IL-6 can be seen in patients with KD.

It was demonstrated that, in general, sera from healthy children contained extremely low levels of IL-6. In marked contrast, elevated levels of serum IL-6 were observed in sera from all patients with acute KD, whereas lower levels of IL-6 were detected in the sera of patients in the subacute phase of the disease. It is of interest whether or not serum IL-6 levels can reflect the severity of the disease. However, no correlation could be seen between the level of IL-6 and coronary involvement in this disease.

Considering the pathophysiological role of IL-6 in the development of KD, it is important to know whether elevated serum levels of IL-6 can be seen exclusively in patients with KD. In this study, increased IL-6 levels could be observed in patients with viral infections. In other studies, increased IL-6 levels can be observed in other diseases (Hirano *et al.* 1990; Kim *et al.* 1991).

These observations suggested that the appearance of IL-6 in the circulation might be a general phenomenon in many inflammatory diseases, whether infectious or not.

It is well known that IL-6 can be produced by a variety of cell types, such as activated T cells, monocytes, fibroblasts, keratinocytes, endothelial cells and epithelial cells (Hirano *et al.* 1990).

Activated T cells can be observed in KD, which can be a source of IL-6 in this disease. The rate of release of sIL-2R appears to depend on the degree of its cell surface expression and is a reflection of lymphocyte activation (Smith 1988). With the result of a high correlation between IL-6 levels and sIL-2R, it can be postulated that the source of IL-6 in patients with KD might be from activated T cells.

However the possibility of another source of IL-6 such as activated endothelial cells, monocytes, etc can not be excluded. Reverse, increased IL-6 in KD could have induced IL-2 and IL-2 receptors which play a role in IL-6. This possibility will require investigation by further studies.

Increased sIL-2R levels are not also an exclusive finding in KD. Rubin and Nelson (1990) reported that the quantitation of sIL-2R, a novel laboratory measure of in-vivo immune system activation, correlates reliably with disease activity in autoimmune inflammatory disorders, transplantation rejection, specific infections, hematologic malignancies and others. Unfortunately, there were not any correlations between increased sIL-2R and coronary artery complications in KD either.

It has been demonstrated that the secreted products of monocytes/macrophages and other cytokines play a key role in modulating the synthesis of acute phase protein (Ritche and Fuller 1983). Highly purified and recombinant preparations of IL-1 and TNF have been shown to alter hepatic synthesis of a number of acute phase proteins (Ganapathi et al. 1988). Recent studies have shown evidence that recombinant preparation of IL-6 can induce hepatic production of the acute phase protein in vivo and in vitro (Gauldie et al. 1987; Castell et al. 1988; Geiger et al. 1988). As shown in Fig. 3, serum levels of IL-6 correlate well with serum concentration of CRP.

It is well known that thrombocytosis can be observed in subacute phase of KD. The pathogenesis of this thrombocytosis in the disease is not established. IL-6 is a well known thrombopoietic factor, which induces the maturation of megakaryocytes (Hirano et al. 1990). Then it can be postulated that thrombocytosis in KD might be from increased IL-6 in the acute phase of the disease. However there was no correlation between peak platelet count during the subacute phase and serum IL-6 levels during the acute phase of the disease. The author could not exclude that systemically damaged endothelium might be a cause of thrombocytosis in KD. Further studies should be done to clarify the cause of thrombocytosis in KD.

In conclusion, studies of IL-6 activity in KD may further understanding of its role in acute phase reactions and in the immune responses of the host.

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