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Increased Serum Interleukin-10 Level in Kawasaki Disease

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Serum IL-10 level in Kawasaki disease(KD) was tested. In the KD patients' sera during the acute phase, the levels of IL-10 were markedly elevated (122.0±39.1 pg/ml) compared to 37±17 pg/ml in the control subjects ($p<0.001$). The serum IL-10 levels remained elevated in the subacute phase (16.7±97 pg/ml, $p<0.001$) and were restored to the normal levels(7.9±3.9 pg/ml) during the convalescent phase. In the patients with acute febrile disease, the serum IL-10 level increased significantly (34.4±14.1 pg/ml, $p<0.001$) compared to that of the age-matched control subjects, but were not as high as in acute phase of KD($p<0.005$). This increase in serum IL-10 levels in KD may contribute to the up-regulation of humoral immunity and to the down-regulation of acute inflammation due to an increase in proinflammatory cytokines.

Key Words: Kawasaki disease, IL-4, IL-10, IL-12

INTRODUCTION

Interleukin-10(IL-10), a 18 kDa polypeptide produced by Th2 helper clones, was originally described as a cytokine synthesis inhibitory factor because it inhibits interferon-γ (IFN-γ) production by Th1 clones. Like IL-6, IL-10 is expressed by a variety of cells, including Th0 and Th1 subsets, monocytes, macrophages, and B cells. IL-10 has a major role in the regulation of immune responses: it blocks the activation of cytokine synthesis and inhibits macrophage, T cell, and NK cell effector functions(Moore *et al.* 1993).

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Human IL-10 has a stimulatory activity comparable to IL-4 on B cells, and together they induce a considerable increase in B-cell proliferation(Moore *et al.* 1993). IL-10 is a differentiation factor for human B cells that are activated by anti-CD40 antibodies, leading to the production of large amounts of IgM, IgG, and IgA(Banchereau *et al.* 1991; Rousset *et al.* 1992).

Kawasaki disease(KD) is an acute systemic vasculitis which predominantly affects infants and young children. Even though the epidemiologic features of this disease suggest that it is related to an infectious agent, the etiology is still obscure.

However, KD is an immunologically interesting disease, because during its course immunological abnormalities including activation of immunocompetent cells can be observed (Leung, 1989). It is also reported that during the acute phase of KD some autoantibodies such as anti type III collagen antibody (Kobayashi *et al.* 1992), antineutrophil cytoplasmic antibody(Guzman *et al.* 1994), IgM autoan-

tibody to vascular endothelial cells activated by interferon-γ (Leung *et al.* 1986), and anti-heat shock protein 65 antibody(Yokoda, 1991) were found. There is a marked increase in circulating B cells that are actively producing immunoglobulins(Leung, 1989), probably the most remarkable immunologic finding in this disease.

There have been some reports of increased serum cytokines during KD, such as IL-1 (Leung *et al.* 1989), IL-2, IFN-γ, TNF-α (Matsubara *et al.* 1990), IL-6(Kim, 1992). In the present study, we asked whether the polyclonal B cell activation which is observed in KD might be caused by an abnormal production of IL-10.

MATERIALS AND METHODS

Patients

Serum samples and venous blood were obtained from 20 patients (12 males) with KD, who were followed at the Severance Hospital, Yonsei University College of Medicine and Sowha Children's Hospital, Seoul, Korea. All patients satisfied at least five of the six criteria for the diagnosis of KD(Kawasaki, 1967). Atypical KD was excluded. The mean age was 2.5 years (ranged from 9 months to 6 years). All patients were treated with intravenously administered gamma globulin(IVGG) in addition to high-dose aspirin therapy. The samples drawn from the patients were tested to determine the immunophenotypes of the lymphocytes and the IL-10 levels at the time of any therapy. Serial samples were obtained from 14 patients subsequently in the subacute phase, and from 10 patients subsequently during the convalescent phase. Samples were obtained from 10 age matched children with acute febrile disease and 10 children who were having routine blood works done before elective surgery. Samples of acute febrile disease samples were collected from the patients before any medications were given. Informed consents were obtained from the parents of the children included in the study.

Analysis of immunophenotypes of peripheral lymphocytes

To determine the phenotype of the peripheral lymphocytes in KD, the cell surface antigens were stained using fluorescein-conjugated (FITC) and/or phycoerythrin(PE)-conjugated anti-Leu-3a(CD4), anti-Leu-1(CD5), anti-Leu-2a (CD8), anti-Leu-12(CD19) monoclonal antibodies, which were purchased from Becton Dickinson Monoclonal Center(San Jose, CA, USA) by the the whole blood method. Briefly, one hundred microliters of the heparinized venous blood was placed in each of 4 labeled tubes. The 100 microliters of monoclonal antibodies and PBS as negative controls were added. The cells were incubated in the dark at 4℃ for 30 min. The red blood cells in each tube were then lysed using 1 mL of lysing reagent. After incubation in the lysing reagent for 30 seconds, the cells were immediately centrifuged at 200 x g for 5 min. The supernatants were removed, leaving approximately 50 microliters of fluid. The pellets were mixed gently with a vortex after adding 3 mL of 0.01M PBS with 2% bovine serum albumin. After centrifugation at 200 x g for 5 min, the supernatant was discarded, leaving approximately 50 microliters of fluid. The pellets were resuspended with 0.3 mL of 1% paraformaldehyde in PBS and were analysed using a FACStar flow cytometer(Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA).

Serum IL-4, IL-10, IL-12 assay

The serum samples were stored at −70℃ until they were tested for serum IL-4, IL-10, and IL-12 levels, which were measured with ELISA kits. IL-4 and IL-10 ELISA kits were purchased from T cell Diagnostics(Woburn, MA, USA) and the IL-12 kit from R & D systems(Minneapolis, MN, USA). The procedure was carried out according to the manufacturer's directions.

Statistical analysis

The students' t test and ANOVA(analysis of variance) were used to analyze the signifi-

cance of the difference between the experimental groups and the normal controls.

RESULTS

Peripheral T cell & B cell compositions in Kawasaki disease

As shown in Table 1, these patients have a decreased T cell percentage, a slight increase in the CD4/CD8 ratio, an increase of B cell percentage, and a decreased CD5+ B cell percentage during the acute phase of disease.

Serum IL-10 level

As shown in Fig. 1, the serum levels of IL-10 were markedly elevated in the KD patients' sera during the acute phase. The acute-phase IL-10 level was 122.0±39.1 pg/ml, compared to 3.7±1.7 pg/ml in the age-matched control subjects ($p<0.001$). The serum IL-10 levels remained elevated in the subacute phase(16.7±9.7 pg/ml, $p<0.001$), even though the patients were afebrile when tested, and decreased to the normal levels during the convalescent phase. In patients with acute febrile disease, the serum IL-10 level increased significantly (34.4±14.1 pg/ml, $p<0.001$) compared to that of the age-matched control subjects. However, these serum levels were not as high as in the acute phase of KD($p<0.005$). The follow-up IL-10 levels during the subacute phase(16.7±9.7 pg/ml) and convalescent phase (7.9±3.9 pg/ml) of KD were significantly lower than the levels measured during the acute phase($p<0.01$)(Fig. 2).

The relationship between the serum IL-10 levels and the percentage of immune cells such as T and B cells was evaluated. There was no statistically significant relationship between these immune cells and the serum IL-10 level(data not shown).

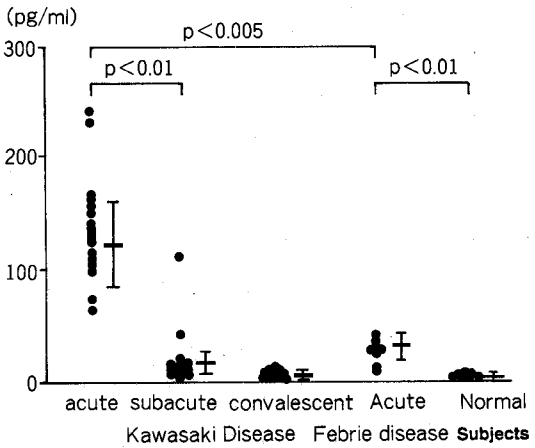

Fig. 1. *Serum interleukin-10 levels in Kawasaki disease during the acute, subacute and convalescent phase, in the patients with acute febrile disease and in normal subjects. Serum interleukin-10 was measured with commercial ELISA kit.*

Table 1. Percentage of T, B and CD5+ cells in Kawasaki disease, acute febrile disease and age-matched normal control

Cell population	Kawasaki(n=20)			Acute[$] febrile disease(n=10)	Normal (n=10)
	Acute	Subacute	Convalescent		
T cell (%)[+,#]	63.5±9.9	69.2±7.9	72.0±7.2	70.5±7.3	73.3±6.9
CD4/CD8[+,*]	2.9±0.4	2.2±0.3	1.5±0.1	3.8±0.8	2.1±0.3
B cell (%)[@,*]	14.8±6.5	13.1±6.0	8.4±3.5	8.9±2.1	9.1±1.7
CD5+B in B(%)[@,*]	15.3±1.5	26.9±2.4	34.9±3.7	36.1±5.0	35.2±3.9

[@]: $p<0.01$ compared with normal controls
[+]: $p<0.05$ compared with normal controls
[#]: $p<0.05$ compared with acute febrile diease
[*]: $p<0.01$ compared with acute febrile disease
[$]: 7-acute tonsillopharyngitis, 3-herpangina

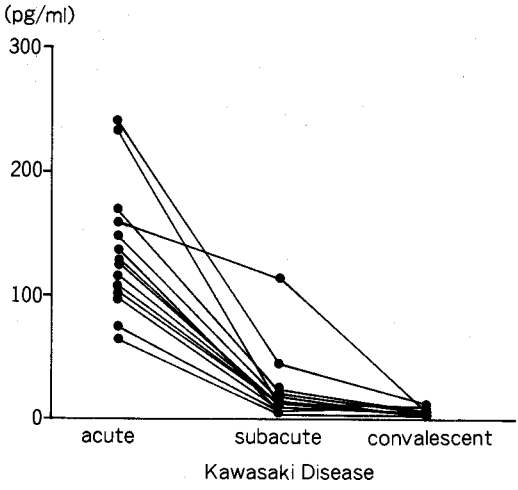

Fig. 2. *Serial changes in serum interleukin-10 levels in Kawasaki disease during the acute, subacute and convalescent phase. Serum interleukin-10 was measured with commercial ELISA kit.*

Serum IL-4 and IL-12 level

We measured the level of serum IL-4, which is produced simultaneously with Il-10 by Th2. We also measured the level of serum IL-12, which is a counterregulatory cytokine of IL-10 in Th1 and Th2 regulation. However, we could not detect a significant amount of either IL-4 or IL-12 in any conditions (data not shown).

DISCUSSION

The underlying pathogenesis of KD remains to be elucidated. The acute phase of KD is often accompanied by the activation of both T cells and B cells(Leung, 1989). The infiltration of macrophages and activated T cells has been observed in vascular lesions of acute KD. Also, the majority of patients with acute KD have been reported to have increased percentages of monocytes that spontaneously secrete abnormally high levels of IL-1(Leung *et al.* 1986) and cause increase in TNF in acute KD(Furukawa *et al.* 1988).

The most remarkable immunologic finding regarding acute KD is an increase in B cells that are actively producing immunoglobulins (Leung, 1989). Moreover, in a previous study, we observed that conventional B cells (CD5−, CD19+) increased, whereas CD5+ CD19+ B cells decreased during the acute phase of KD (Kim *et al.* 1996). We confirmed these B cell findings in this study.

Usually, B cells stimulated by membranes from activated T cells proliferate and differentiate to secrete immunoglobulins in the presence of cytokines such as IL-4 and IL-5 from Th2 cell clones. Th2 cell clones also produce IL-10 in addtion to IL-4 and IL-5. IL-10 inhibits the production of cytokines by Th1 clones in response to antigens. In addition, it has been demonstrated that IL-10 has an immunostimulatory effect on B cells as up-regulation of MHC class II antigens and increases the proliferation of B cells and production of immunoglobulins(Moore *et al.* 1993). IL-10 is a differentiation factor for human B cells activated by anti-CD40 antibodies causing production of large amounts of IgM, IgG, and IgA(Burdin *et al.* 1995). We postulated that serum IL-10 levels may increase in KD, in which polyclonal B cell activation was observed. As expected, the level of serum IL-10 increased during the acute phase of KD and decreased during the subacute phase. The significance of increased IL-10 level observed in KD is not known, but the increased B cell activation and hypergammaglobulinemia observed during the acute phase of KD may be caused in part by increased serum IL-10 levels.

IL-10 is produced by LPS-stimulated CD5+ B cells. Moreover, it was suggested that IL-10 may be an autocrine factor that is essential for CD5+ B cell development(Kantor, 1991). In our results, the percentage of CD5+ B cells in total B cells decreased markedly during the acute phase of KD and increased to the normal level during the convalescent phase, resulting perhaps from an autocrine function of IL-10. We think that the production of IL-10 increases in order to counteract the decrease in CD5+ B cells during the acute phase of KD.

Increased IL-10 levels have been observed in

experimental parasitic infections such as *Trypanosoma cruzi* and *Schistosoma mansoni*, mycobacterial infection, leishmaniasis, HIV infection(de Vries and de Waal Malefyt, 1995) and atopic dermatitis(Ohmen *et al.* 1995). To this, we add the interesting finding that the serum IL-10 level increases in KD as well.

IL-10 can be produced by a variety of cells including Th2, Th0, Th1 subsets, monocytes, macrophages, and B cells(Moore *et al.* 1993). It has been demonstrated that in KD T cells, B cells, and monocytes were activated, any of which could be a cellular source of IL-10. However, the cellular source of IL-10 in KD is not yet known and we should conduct further study on the cellular source of IL-10.

Th2 clones produce IL-4 in addition to IL-10. Like IL-10, IL-4 can promote the growth and differentiation of B cells(Paul, 1990). Contrary to our expectation that IL-4 levels would also increase during acute KD to stimulate circulating B cells which produce immunoglobulins, we observed serum IL-4 levels in KD during the acute and subacute phases not to have increased. Although we cannot fully explain this discrepancy between IL-4 and IL-10 levels in the acute KD, we suggest two possible explanations. First, Th2 cells might not be the cellular source of IL-10. Second, activated Th2 cells in KD might produce only IL-10, not IL-4. This is suggested by the fact that IL-10 is not coordinatively elevated along with other Th2 cytokines(Lu *et al.* 1995). And the differential regulation of IL-4 and IL-10 has also been shown in activated T cell clones in which elevations in IL-4 gene expression are cyclosporin sensitive whereas IL-10 elevations are cyclosporin resistant(Wang *et al.* 1991).

IL-10 also inhibits monocyte/macrophage function. This inhibition of monocyte/macrophage APC function by IL-10 affects both cytokine synthesis and the proliferation of human T cells(de Waal Malefyt *et al.* 1991). This effect may be partially due to the down-regulation of monocyte/macrophage class II MHC antigens. It was thought that increased TNF-α levels in the acute KD sera was caused by activated monocytes. It was reported that IL-10 inhibits the release of TNF-α from monocytes through the down-regulation of TNF-R and the increased release of soluble TNF-R(Joyce *et al.* 1994). So it may be speculated that the increased level of IL-10 in the acute KD sera might serve to inhibit activated monocytes, resulting in the decrease of TNF-α production.

IL-12 can augment the proliferation of PHA-activated lymphoblasts, including activated T cells of the CD4+ and CD8+ subsets (Gately *et al.* 1991), independently of IL-2. IL-12 stimulates the production of IFN-γ from T cells and NK cells(Kobayashi *et al.* 1989; Chan *et al.* 1991). In KD, activated T cells and increased levels of serm IFN-γ have been demonstrated. We expected that the level of serum IL-12 in acute KD would also increase. However, we observed no increase in serum IL-12 levels in acute and subacute KD.

In conclusion, serum IL-10 levels in KD during the acute phase markedly increased. This increase in serum IL-10 levels may contribute to the up-regulation of humoral immune responses and to the down-regulation of acute inflammation from an increase in proinflammatory cytokines such as TNF-α.

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