

Decreased CD5+B Cells during the Acute Phase of Kawasaki Disease

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We investigated the changes of CD5+B cells in the peripheral blood of 20 Kawasaki disease (KD) patients. The percentage of CD5+B cells in the total lymphocytes and in the total B cells significantly decreased during the acute phase of KD ($p < 0.01$), compared to that in the age-matched normal control subjects. After intravenous immunoglobulin (IVIG) treatment, the percentage of CD5+B cells increased, but was still lower than that in the normal controls ($p < 0.01$). During the convalescent phase of the disease, the percentage of CD5+B cells was restored to the normal levels. The levels of CD5+B cell percentage in the total B cells of the patients with acute febrile disease showed similar levels to age-matched normal controls. The decreased CD5+B cells in the patients with KD provides an additional abnormal immunological finding during the acute phase of the disease.

Key Words: CD5+B cell, Kawasaki disease, IVIG

Kawasaki disease (KD) is an acute febrile illness that primarily affects infants and young children. The major clinical features of acute KD are prolonged fever of more than 5 days' duration that is nonresponsive to antibiotics or antipyretics; bilateral conjunctival injection; enlarged cervical lymph nodes; induration and erythema of the hands and feet; it also presents with inflammation of lips, tongue, and oropharynx, and polymorphous skin rashes. Although KD is usually self-limited, with resolution of the acute clinical symptoms, serious complications such as coronary artery aneurysms or ectasia can occur. KD has now become one of the leading causes of acquired heart disease in the pediatric age group (Kawasaki, 1967).

Despite the excellent clinical descriptions, its pathogenesis remains to be clarified. Recently, toxic shock syndrome toxin-1 producing *Staphylococcus aureus* was suggested as an etiological agent of this disease (Leung *et al.* 1993), but there is controversy surrounding the matter.

Many authors have been interested in the possibility that immunoregulatory abnormalities play an important role in the pathogenesis of this disease. During the acute phase of KD, there is an immunoregulatory T cell imbalance such as absolute T cell lymphopenia, both in the helper and suppressor T cells. Conversely, there is a marked increase in the circulating B cells that are actively producing immunoglobulins, which is probably the most remarkable immunologic finding in this disease (Leung *et al.* 1982). It is also reported that during the acute phase of KD, some autoantibodies such as autoantibody to type III collagen (Kobayashi *et al.* 1992), antineutrophil cytosolic antibody (Dillon and Tizard, 1991), IgM autoantibody to vascular endothelial cells activated by interferon- γ (Leung *et al.* 1986) and

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anti-heat shock protein 65 antibody(Yokoda *et al.* 1993) were found. Therefore KD may be understood as an autoimmune disease.

The CD5+ molecule is a 67 kDa glycoprotein initially thought to be exclusive to the T cell(Canto and Boyse, 1975). Hayakawa *et al.* (1983) originally distinguished a subset of mouse B cells that bear low levels of the pan-T cell glycoprotein Ly-1(CD5). Evidence of the existence of a CD5-bearing B cell subset in humans was reported by Boumsell *et al.*(1987). They reported that T cell markers were shared by B cells in some patients with chronic lymphocytic leukemia(Schroeder and Dighiero, 1994). Also there is an increased number of circulating CD5+ B cells in several autoimmune diseases(Youinou *et al.* 1993).

KD is a kind of rheumatic disease, and a marked increase in circulating B cell is a remarkable finding during the acute phase of KD. However, the changes in CD5+B cell population has not been studied. Here, we have investigated the circulating CD5+ B cells in patients with KD during the acute, subacute and convalescent phases of the disease. The circulating CD5+ B cells decreased during the acute phase of KD and were restored to normal levels during the convalescent phase.

MATERIALS AND METHODS

Patients

Venous blood was obtained from 20 patients (12 males) who were followed up at the Severance Hospital, Yonsei University College of Medicine and Sowha Children's Hospital, in 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 immune globulin(IVIG) in addition to a high-dose of aspirin therapy. The samples drawn from the patients were tested to determine the immunophenotypes of the lymphocytes at the time of any therapy. Serial samples were ob-

tained from 16 patients subsequently in the subacute phase, and from 15 patients subsequently during the convalescent phase. Samples were obtained from 10 age matched children with acute febrile disease (acute tonsillopharyngitis) and 10 children who were having routine blood work done before elective surgery. Acute febrile disease samples were collected from the patients before any medications were given. An informed consent was 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 by the the whole blood method(Pamela *et al.* 1986). These were purchased from Becton Dickinson Monoclonal Center (San Jose, CA, USA). 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°C for 30 min. The red blood cells in each tube were then lysed using 1mL of a lysing reagent. After incubation in the lysing reagent for 30 seconds, the cells were immediately centrifuged at 200×g for 5 min. The supernatants were removed, leaving approximately 50 microliters of fluid. The pellets were mixed gently with a vortex after adding 3mL of 0.01 M PBS with 2% bovine serum albumin. After centrifugation at 200×g for 5 min, the supernatant was discarded, leaving approximately 50 microliters of fluid. The pellets were resuspended in PBS and were analysed using a FACStar flow cytometer(Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA).

Statistical analysis

The Students' t test and ANOVA(analysis

Table 1. Peripheral blood pictures of the patients with Kawasaki disease during the acute, subacute, and convalescent phase, of the patients with acute febrile disease and age-matched normal controls

Cell population	Kawasaki(n=20)			Acute febrile disease(n=10)	Normal (n=10)
	Acute	subacute	Convalescent		
WBC(/mm ³)	13,975±2,531*	11,121±2,853	8,554±1,744	12,151±2,154	8,521±1,514
Lymphocyte(%)	30.7±14.1**	45.4±13.2	45.8±12.1	43.0±15.5	46.9±12.5
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±2.1**	13.1± 6.0	8.4± 3.5	8.9± 2.1	9.1± 1.7

*: $p<0.01$ compared with normal controls, +: $p<0.05$ compared with normal controls

**: $p<0.05$ compared with acute febrile disease, *: $p<0.01$ compared with acute febrile disease

of variances) were used to analyze the significance of the difference between the experimental groups and the normal controls.

RESULTS

As shown in Table 1, peripheral white blood cell counts increased significantly during the acute phase of KD($p<0.01$), which decreased after IVIG treatment and returned to normal level during the convalescent phase of KD. The proportions of peripheral lymphocytes to white blood cell counts decreased during the acute phase of KD($p<0.05$), to a level of a lymphopenia. Lymphocyte counts returned to the normal level after IVIG infusion. Also, these patients showed a decreased T cell count($p<0.05$) with an increased CD4/CD8($p<0.05$), and an increased B cell count($p<0.01$) during the acute phase of disease. These findings were normalized during the convalescent phase of disease.

The levels of CD5+ B cell percentages in the total lymphocyte counts were markedly decreased in the KD patients during the acute phase, in which the mean±SD was $5.8\pm4.9\%$, compared to $16.7\pm4.1\%$ in the age matched control subjects($p<0.01$). The levels of CD5+ B cell percentages in the total lymphocytes remained significantly decreased in the subacute phase (mean $8.4\pm4.2\%$; $p<0.05$), even though the patients were afebrile when tested.

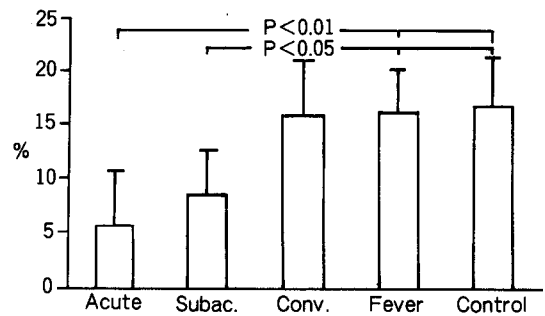


Fig. 1. The percentage of CD5+ B cells out of the total lymphocytes in patients with Kawasaki disease during the acute (Acute), subacute (Sub) and convalescent (Conv); patients with acute febrile disease (Fever) and normal controls (Control).

In the patients with acute febrile disease, the levels of CD5+ B cell percentages in the total lymphocytes were similar(mean $16.1\pm4.1\%$) to the age-matched control subjects(Fig. 1).

As shown in Fig. 2, the levels of CD5+ B cell percentages in the total B cells showed similar results as those in the total lymphocytes. The CD5+ B cell percentages in the total B cells were $16.4\pm8.7\%$ during the acute phase of KD($p<0.01$), compared to normal control subjects(mean $42.2\pm9.8\%$). The levels of CD5+ B cell percentages in the total B cells remained significantly decreased in the subacute phase (mean $27.4\pm10.2\%$; $p<0.05$), even though the patients were afebrile when tested. In the patients with acute febrile disease, the

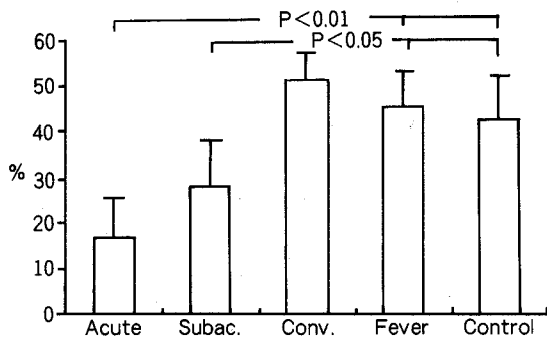


Fig. 2. The percentage of CD5+ B cells out of the total B cells in patients with Kawasaki disease during the acute (Acute), subacute (Sub) and convalescent (Conv); patients with acute febrile disease (Fever) and normal controls (Control).

levels of CD5+ B cell percentages in the total B cells were similar (mean $45.0 \pm 7.9\%$) to the age-matched control subjects (Fig. 2).

The follow-up levels of CD5+ B cell percentages in the total B cells during the subacute phase of KD (mean $27.4 \pm 10.2\%$) were significantly higher than the levels measured during the acute phase (mean $16.4 \pm 8.7\%$; $p < 0.05$). And during the convalescent phase of KD, the levels of CD5+ B cell percentages in the total B cells (mean $51.3 \pm 5.9\%$) seemed to be higher than the levels in normal controls, but it was statistically nonsignificant ($p > 0.05$) (Fig. 2, 3).

DISCUSSION

The etiology of KD remains to be elucidated. The acute phase of KD is often accompanied by an increased number of DR+CD3+ and DR+CD4+ T and a decreased number of CD8+ T cells (Leung, 1989). Recently, the same picture was observed in the small intestinal mucosa in KD (Nagata *et al.* 1993). In addition, it has been reported that most patients with acute KD have increased percentages of monocytes with spontaneous secretion of abnormally high levels of IL-1 (Maury *et al.* 1988). An increase in serum TNF- α (Furukawa *et al.*

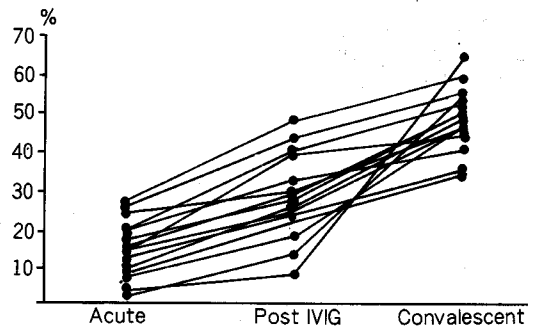


Fig. 3. Levels of CD5+ B cells in serial samples obtained from 14 patients during the acute phase of KD (Acute) and subsequently during the subacute (Post IVIG) and convalescent phase (Convalescent) of their illness.

1988) and IL-6 (Kim, 1992) has also been described in these patients. Besides activation of T cells and monocytes, there is a marked increase in circulating B cells that are actively producing immunoglobulins (Leung *et al.* 1982). An increase of B cells in peripheral blood is probably the most remarkable immunological finding in this disease. It has been also reported that autoantibodies such as an antibody to type III collagen (Kobayashi *et al.* 1992), antineutrophil cytosolic antibody (Dillon and Tizard, 1991), and IgM antibody to vascular endothelial cells activated by IFN- γ (Leung *et al.* 1986) have been observed during the acute phase of KD. With these findings, many authors have speculated that KD is an autoimmune systemic vasculitis.

Recently, increases in CD5+ B cell frequency have been reported in patients suffering from autoimmune diseases, such as rheumatoid arthritis, Sjögren's syndrome, myasthenia gravis, insulin-dependent diabetes mellitus and Hashimoto's thyroiditis (Youinou *et al.* 1993). Whether these increases are due to expansion of CD5+ B cell lineage cells in the human or due to activation-induced expression of CD5 by conventional B cells is unclear.

Also the potential role of CD5+ B cells in autoimmunity is still controversial. The hybridomas produced with CD5+ B cells from non-immunized newborn (Dighiero *et al.* 1985)

or adult mice(Dighiero *et al.* 1983) show autoreactivity and polyreactivity. It was reported that CD5+B cells in the patients with rheumatoid arthritis produced an IgM-rheumatoid factor. IL-4 downregulates the surface expression of the CD5 molecule on B cells and inhibits spontaneous immunoglobulin and rheumatoid factor(Hidaka *et al.*, 1992). However, others reported that the IgM rheumatoid factor is unlikely to be derived solely from CD5+ B cells(Xu *et al.* 1994).

It is well documented that an increase of B cells in the peripheral blood of patients with KD is a remarkable finding. However, the changes of CD5+ B cells in acute KD has not been reported. KD is thought to be an autoimmune disease. For these reasons, we have extended our interest in changes of CD5+ B cells during the acute phase of KD. Contrary to our expectation, the CD5+ B cells during the acute phase of KD decreased significantly. But CD5+ B cells showed a significant increase after IVIG treatment, and were restored to a normal level during the convalescent phase of the disease. For controls, the patients with acute febrile disease did not show a decreased CD5+ B cells.

It cannot be explained what the cause of decreased CD5+ B cells during the acute phase of KD might be. Iciek *et al.*(1994) reported the decreased percentage of CD5+ B cells in three murine models of systemic autoimmune disease: murine acquired immunodeficiency syndrome; chronic graft-versus-host disease; and collagen-induced arthritis. They explained that the apparent decline in CD5+ B cell frequency was due to increases in either T cells, conventional epsilon R+ B cells, or both. However, in our study, the acute phase of KD showed a decrease of T cells. Therefore it does not seem that decreased CD5+ B cells was due to increases in the T cells. Also, a decrease of CD5+ B cells was not due to an increase in conventional B cells, because changes of CD5+ B cells in acute KD showed a decrease of absolute CD5+ B cells. So a decrease of CD5+ B cells in KD does not seem to be from the changes of T and B cells.

A major breakthrough in the treatment of KD was the introduction of high-dose IVIG,

which is very effective in reducing the systemic symptoms and the prevalence of coronary artery abnormalities, which is the main complications of KD during the convalescent phase(Furusho *et al.* 1984). Most of KD patients become afebrile immediately after the first infusion of IVIG. This means that IVIG may influence the immune system rather than a simple neutralization of the offending agent mediating KD. This evidence was supported by recovery of immunoregulatory abnormalities in KD after IVIG treatment. IVIG treatment produced an increase in the percentage of CD3+ T cells, CD4+ T cells and CD8+ T cells. Furthermore, there was a highly significant decrease in spontaneous IgG and IgM production by B cells after IVIG treatment in KD(Leung, 1989).

With these results, the mechanism of IVIG in KD was thought to be an effect of reduction in antibody-producing B cells. In our study, the conventional B cells showed a significant decrease after IVIG. It could be observed that CD5+ B cells increased after IVIG treatment. Recently, it has been reported that CD5+ B cells suppress the pokeweed mitogen (PWM)-stimulated immunoglobulin synthesis of the normal peripheral lymphocytes(Paglieroni *et al.* 1988). It can be speculated that the decrease of CD5+ B cell in acute KD may increase the immunoglobulin synthesis from conventional B cells, which can be observed in acute KD. Increase of CD5+ B cell after IVIG may inhibit an increased immunoglobulin synthesis of conventional B cells.

We might propose that the initiation of immunological abnormality of the conventional B cell in KD was preceded by a decrease of CD5+ B cells, and the recovery of conventional B cells after IVIG treatment was also preceded by an increase of CD5+ B cells. And the action mechanism of IVIG in KD may be directed on CD5+ B cells.

We observed the correlation between CD5+ B cells and CD4+T, CD8+T, and B cells. However there were no meaningful correlations between these groups(data not shown).

In conclusion, a significant decrease of CD5+ B cell during the acute phase of KD is an

additional abnormal immunological finding. Kawasaki disease may not be a disease of the T cell but of the B cell, especially of CD5+ B cell.

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