ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2001) 19: 85-91

Inverse Correlation between Macrophage-Colony Stimulating Factor, Cholesterol and High Density Lipoprotein Cholesterol in Kawasaki Disease

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SUMMARY **Kawasaki disease (KD) is a childhood-onset vascular disease. We assessed the concentrations of macrophage-colony stimulating factor (M-CSF) and those of lipids in sera from patients with KD. The M-CSF concentration in patients with acute-phase KD was 2,914 ± 159 U/ml, significantly higher than that in control subjects with infectious diseases (1,241 ± 96 U/ml). The elevated levels of this cytokine in the acute phase fell to 1,319 ± 138 U/ml in the convalescent phase. Total and high-density lipoprotein cholesterol concentrations in acute phase KD (113.8 ± 8.4 and 21.5 ± 2.3 mg/dl, respectively) were lower than in the infectious disease controls (195.8 ± 7.0 and 62.5 ± 1.8 mg/dl). The elevation of M-CSF correlated with the decrease of total and high-density lipoprotein cholesterol. Overproduction of macrophage-colony stimulating factor activates macrophages and monocytes and may disturb the lipid metabolism. Both effects could contribute to vasculitis in KD.**

Although Kawasaki disease is among the most common vascular diseases affecting children in Japan, its etiology remains obscure. Immunologic analyses implicate activation of monocytes and macrophages in the etiology of the disease.[1]

Macrophage-colony stimulating factor is a cytokine that stimulates formation of macrophage colonies by functionally activating mature macrophages and monocytes.[2] This cytokine also influences the lipid metabolism, attenuating the concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol.[3,4] Although elevated levels of M-CSF in Kawasaki disease have been documented,[5] lipid metabolism in Kawasaki disease has not been definitely related to this cytokine.

In the present study, we measured serum concentrations of this cytokine before and after treatment of Kawasaki disease, examining the correlation between the cytokine and disease activity. To assess cause-and-effect relationships between M-CSF and lipid metabolism, total and HDL cholesterol levels were also evaluated.

SUBJECTS AND METHODS

Subjects

Subjects included six patients with Kawasaki disease (two males, four females), ranging in age from 5 months to 4 years and 6 months (median age, 2 years and 6 months; Table 1) and six disease controls (three males, three females), with an age range from 11 months to 3 years and 1 month (median age, 2 years). The diagnosis was based on the fourth version of criteria established by the study group of the Japanese Ministry of Public Welfare (1982). The diagnostic criteria require at least five of the six characteristic clinical features of this illness: 1)

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Table 1 Patients with Kawasaki disease

Subject number	Age	Sex	γ-globulin therapy	Other therapy	Cardiac involvement
1	10 months	male	550 mg/kg/day, 5d	Flurbiprofen	Mitral insufficiency
2	11 months	female	400 mg/kg/day, 5d	Aspirin	None
3	4 years 6 months	female	390 mg/kg/day, 5d	Flurbiprofen	None
4	1 year 1 month	female	420 mg/kg/day, 5d	Aspirin	Reduced EF[1]
5	10 months	female	490 mg/kg/day, 5d	Flurbiprofen	None
6	5 months	male	400 mg/kg/day, 5d	Aspirin	None

[1]ejection fraction

Table 2 Disease controls

Subject number	Age	Sex	Symptoms					Diagnosis
			Cervical			Hyperemia		
			Fever	Lymph node	Eruption	Conjunctiva	Lips	
1	1 year 5 months	male	+	+	+	-	-	Bacterial pneumonia
2	1 year 10 months	female	+	+	+	-	-	*Mycoplamsma* pneumonia
3	3 years 1 month	female	+	+	+	+	-	*Mycoplasma* pnuemonia
4	1 year 2 months	female	+	+	+	+	-	*Mycoplasma* pnuemonia
5	11 months	male	+	+	+	-	-	Viral meningitis
6	1 year 1 month	male	+	+	+	+	+	Measles

the presence of fever for at least 5 days, 2) bilateral bulbar conjunctival injection, 3) changes in the mucosa of the oropharynx, including injected pharynx, injected and/or dry fissured lips, strawberry tongue, 4) changes of the peripheral extremities, such as edema and/or erythema of the hands or feet in the acute phase; or periungual desquamation in the subacute phase, 5) rash, primarily truncal; polymorphous but nonvesicular, 6) cervical adenopathy, > 1.5 cm, usually lymph-adenopathy.

The disease controls included patients with other diagnoses who had fever lasting more than 5 days and a skin eruption, cervical lymphadenopathy, or hyperemia of the bulbar conjunctiva or lips, resembling findings in Kawasaki disease (Table 2). Etiologic agents in these patients were identified by bacterial culture or detection of antibodies against causative viruses.

Blood samples were obtained at day 5 before gamma globulin therapy and one to two days after the cessation of the gamma globulin treatment in all KD cases. Also the control cases had blood withdrawn in the acute phase. Monocyte counts, serum M-CSF, and serum lipids were analyzed. Informed consent was obtained from each subject's parents/guardians prior to enrollment in this study.

Treatment

The patients with KD were treated with intravenous gamma globulin (400 to 540 mg/kg/day) for 5 consecutive days together with daily oral antiinflammatory drugs.

Monocyte counts in the peripheral blood

The numbers of the monocytes identified by Wright staining were counted under the microscopy.

Measurements of serum M-CSF

M-CSF was measured by sandwich enzyme-linked immunosorbent assay (ELISA)[6] using horse anti-human M-CSF antibody (Mclean, VA) and rabbit anti-human M-CSF antibody (BLMCP, Genzyme, Cambridge). Thereafter, the reaction was performed with peroxidase-labeled goat anti-rabbit IgG (Kirkegaad & Perry Laboratories, Inc., Gaithersberg, MD).

Measurements of serum cholesterol

Serum levels of total cholesterol and HDL-cholesterol were determined by POD aniline methods[7] and selective inhibition,[8] respectively.

Statistical analysis

Mann-Whitney test was used for the analysis of statistical significance. Statistical significance was defined as a $p < 0.05$.

RESULTS

Peripheral blood monocytes

Numbers of peripheral blood monocytes were 916 ± 190/μl in the acute phase of KD which were significantly higher than those in the control group (263 ± 35/μl, $p < 0.001$; Fig. 1).

M-CSF

Serum concentrations of M-CSF levels in acute-phase KD were 2,914 ± 159 U/ml, higher than

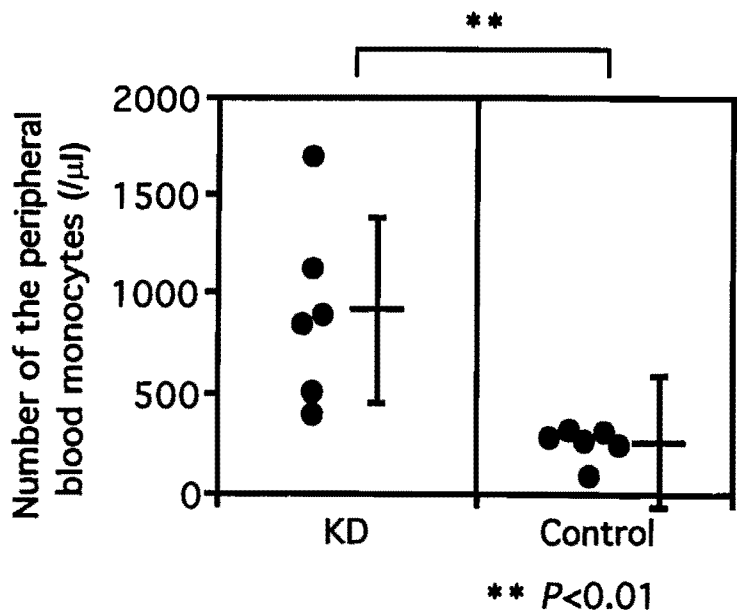

Fig. 1 Comparisons of the numbers of peripheral blood monocytes between patients with KD and disease controls. Data are means ± standard error. ** $p < 0.01$.

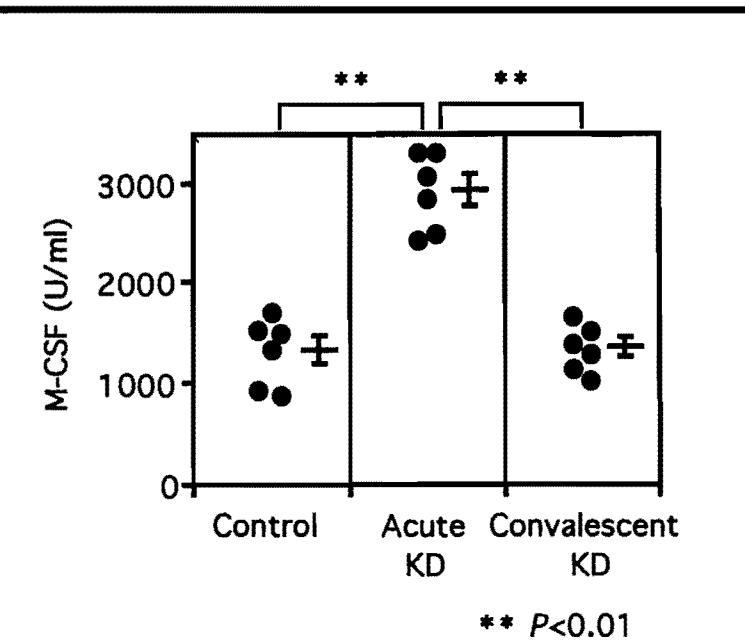

Fig. 2 Serum M-CSF in acute and convalescent phases of KD compared with those in disease-control subjects. Data are means ± standard error. ** $p < 0.01$.

those in controls (1,341 ± 96 U/ml, $p < 0.001$). The acute-phase elevation subsided in the convalescent phase after treatment with gamma globulin (1,319 ± 138 U/ml, $p < 0.001$; Fig. 2). Serum M-CSF correlated with peripheral blood monocyte counts (Fig. 3).

Total cholesterol

Serum total cholesterol concentrations in acute-phase KD were 113.8 ± 8.4 mg/dl, lower than in controls (195.8 ± 7.0 mg/dl; $p < 0.001$). Total cholesterol increased to 174.4 ± 6.7 mg/dl after therapy ($p < 0.05$; Fig. 4a). Total cholesterol correlated inversely with M-CSF (Fig. 4b).

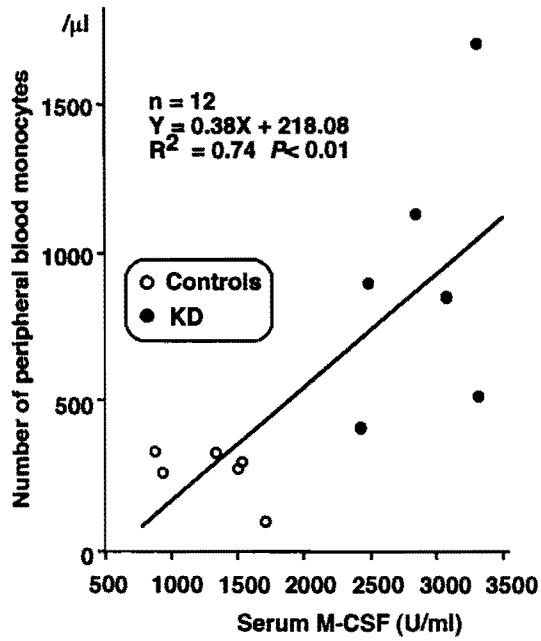

Fig. 3 Correlation of M-CSF with peripheral blood monocyte counts. Patients with KD in the acute phase and disease controls are shown.

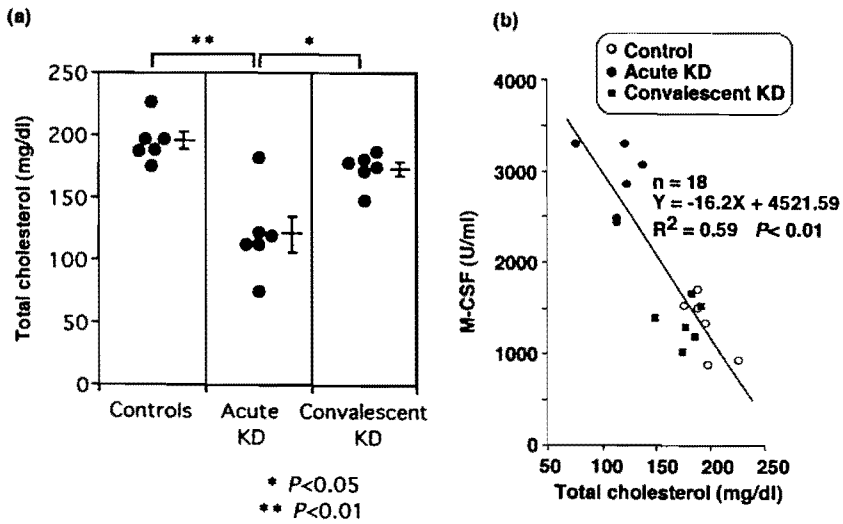

Fig. 4 Serum concentrations of total cholesterol in acute and convalescent phases of KD and in control subjects. (a) Total cholesterol in controls, acute phase and convalescent phase. Data are means ± standard error. *$p < 0.05$, **$p < 0.01$. (b) An inverse correlation is shown between total cholesterol and serum M-CSF.

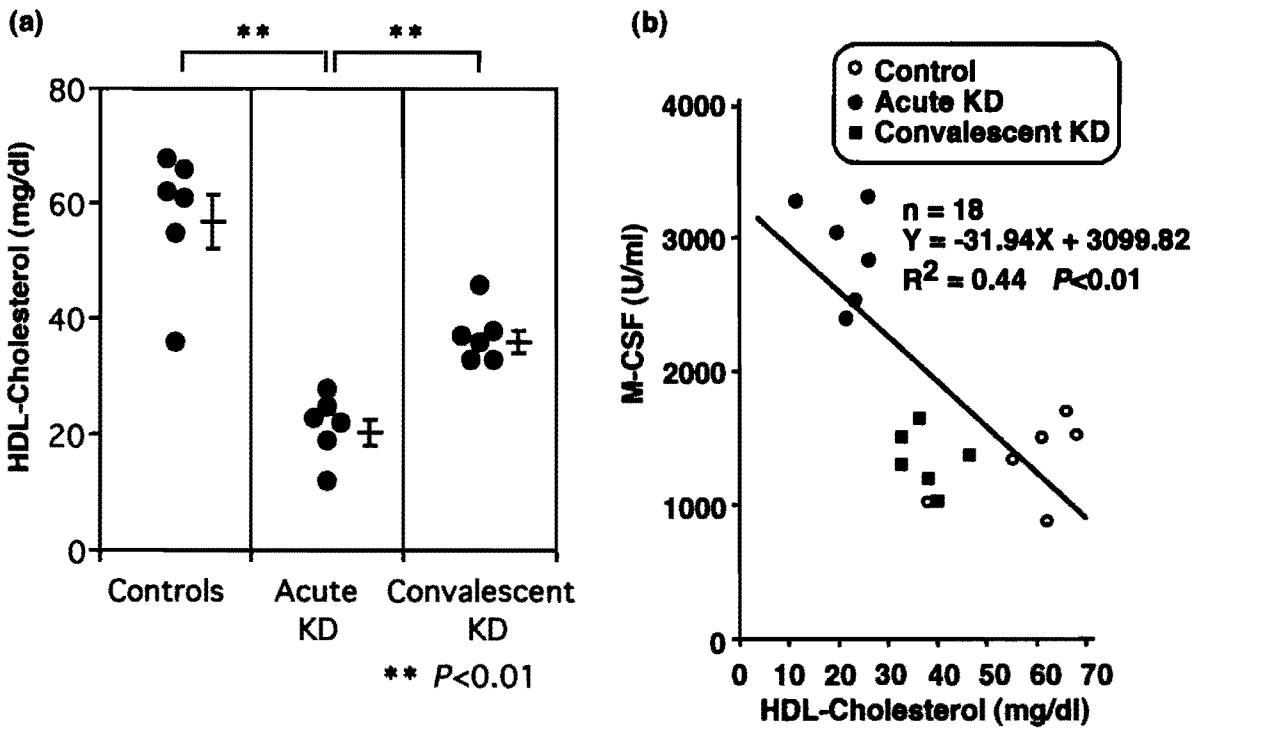

Fig. 5 Serum concentrations of HDL cholesterol in acute and convalescent phases of KD and in control subjects. (a) HDL cholesterol in controls, acute phase and convalescent phase of KD. Data are means ± standard error. $^{**}p < 0.01$ (b) Inverse correlation between HDL cholesterol and serum M-CSF.

HDL cholesterol

Patients with KD in the acute phase showed lower levels of HDL cholesterol (21.5 ± 2.3 mg/dl) than the control group (62.5 ± 1.8 mg/dl; $p < 0.001$). Treatment increased the low HDL cholesterol to 35.2 ± 3.0 mg/dl after therapy ($p < 0.05$; Fig. 5a). Levels of HDL cholesterol correlated inversely with serum M-CSF (Fig. 5b).

DISCUSSION

Diagnosis of the KD requires differentiation from other diseases which presenting with similar symptoms. We compared M-CSF and lipid metabolites of patients with KD to those of control subjects with bacterial pneumonia, *Mycoplasma* pneumonia, viral meningitis, and measles who showed somewhat similar symptoms. Although both groups shared some clinical features, only patients with KD showed significant elevations of M-CSF in their sera. In concordance with the upregulation of this cytokine, numbers of peripheral blood monocytes were increased in KD, suggesting activation of monocytes and macrophages by the elevated M-CSF.

Increased M-CSF normalized with administration of intravenous gamma globulin, accompanied by clinical improvement. Thus M-CSF serum levels reflect disease activity similar to interleukin (IL)-1β [9] tumor necrosis factor (TNF)-α [9,10,11,12,13] and IL-6.[9,14]

One of the most important sequelae of KD is cardiac involvement. Our study included one patient with transient reduction of the ejection fraction and another with transient mitral insufficiency. Since these two subjects did not show higher concentrations of M-CSF than other patients, we could not link the cytokine directly to cardiac involvement, though future study of larger numbers of subjects are needed to evaluate such a connection more in depth.

Previous reports concerning serum cytokine behaviors in KD studied interferon (IFN)-γ,[9,15] monocyte chemoattractant protein-1,[16] IL-1β, [9] IL-2,[9] IL-4,[17] IL-6,[9,14] IL-8,[9,14] IL-10,[9,17,18] TNF-α,[9,10,11,12,13] granulocyte colony-stimulating fac-

tor (G-CSF)[9] and transforming growth factor-β1.[19] TNF-α appears to be a key cytokine in the disease and has been implicated in coronary artery involvement.[11] This cytokine has been suggested to promote activation of monocytes and macrophage. Macrophages appear to play a central role in the pathogenesis of KD.[20] Excessive TNF-α in KD may also result from the activation of macrophages by high serum levels of M-CSF, based on the findings that TNF-α is secreted by activated monocytes and macrophages. Further analyses to clarify this issue are in progress.

While a previous report by Igarashi *et al.*[5] already suggested an elevation of M-CSF in acute-phase KD, this cytokine has to date not been studied in relation to an abnormal lipid metabolism in this illness. This cytokine has been shown to reduce the total serum cholesterol by affecting the lipid metabolism.[3,4] Our present results demonstrate an inverse relationship between M-CSF and total cholesterol and HDL-cholesterol in KD. Thus, an aberrant lipid metabolism in KD may be ascribed to the up-regulation of this cytokine.

Lipid metabolites including oxidized low density lipoproteins were increased in the serum of patients with KD.[21] *In vitro* such metabolites may induce oxidative damage of vascular endothelium.[22] Because cytotoxicity from LDL has been shown to correlate inversely with serum HDL,[22] the reduction of HDL as shown in this study may accelerate vascular lesions in the disease.

Accumulation of macrophages in coronary arteries occurs in the acute phase of KD.[23] Such macrophages oxidize LDL upon contact. Should macrophages activated by M-CSF come into contact with this lipoprotein, initiation or potentiation of endothelial damage in the coronary arteries could result.

The present data suggest that increased M-CSF is related to the induction of vasculitis in KD, making the cytokine a useful marker in the evaluation of vasculitis. The elevation of M-CSF in the case of KD without coronary involvement may stress the notion that KD is a vascular disease. The hematologic abnormalities, aberrant lipid metabolism, and vasculitis commonly observed can be explained by the kinetics of M-CSF in serum. Further analysis to clarify mechanisms by which this cytokine is produced and carries out its actions may improve the pathophysiologic understanding of the disease and suggest new therapeutic strategies.

REFERENCES

1. Furukawa S, Matsubara T, Okumura K, Tabuta K. Decreased expression of CD23 on peripheral blood macrophages/monocytes during acute Kawasaki disease with coronary artery lesions. Int Arch Allergy Immunol 1993; 102: 335-9.
2. Motoyoshi K, Takaku F, Mizoguchi H, Miura Y. Purification and some properties of colony-stimulating factor from normal human urine. Blood 1978; 52: 1012-20.
3. Motoyoshi K, Takaku F. Serum cholesterol-lowering activity of human monocytic colony-stimulating factor [letter]. Lancet 1989; 2: 326-7.
4. Shimano H, Yamada N, Ishibashi S, *et al.* Human monocyte colony-stimulating factor enhances the clearance of lipoproteins containing apolipoprotein B-100 via both low density lipoprotein receptor-dependent and -independent pathways in rabbits. J Biol Chem 1990; 265: 12869-75.
5. Igarashi H, Hatake K, Tomizuka H, Yamada M, Gunji Y, Momoi MY. High serum levels of M-CSF and G-CSF in Kawasaki disease. Br J Haematol 1999; 105: 613-5.
6. Hanamura T, Motoyoshi K, Yoshida K, *et al.* Quantification and identification of human monocytic colony-stimulating factor in human serum by enzyme-linked immunosorbent assay. Blood 1988; 72: 885-92.
7. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20: 470-5
8. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977; 62: 707-14.
9. Yoshioka T, Matsutani T, Iwagami S, *et al.* Polyclonal expansion of TCRBV2- and TCRBV6-bearing T cells in patients with Kawasaki disease. Immu-nol 1999; 96: 465-72.
10. Maury CP, Salo E, Pelkonen P. Elevated circulating tumor necrosis factor-alpha in patients with Kawasaki disease. J Lab Clin Med 1989; 113: 651-4.
11. Furukawa S, Matsubara T, Motohashi T, Tsuda M, Sugimoto H, Yabuta K. Immunological abnormalities in Kawasaki disease with coronary artery lesions. Acta Paediatr Jpn 1991; 33: 745-51.
12. Sakaguchi M, Kato H, Nishiyori A, Sagawa K, Itoh K. Characterization of CD4+ T helper cells in patients with Kawasaki disease (KD): preferential production of tumour necrosis factor-alpha (TNF-alpha) by V beta 2- or V beta 8- CD4+ T helper cells. Clin Exp Immunol 1995; 99: 276-82.
13. Eberhard BA, Andersson U, Laxer RM, Rose V, Silverman ED. Evaluation of the cytokine response in Kawasaki disease. Pediatr Infect Dis J 1995; 14: 199-203.
14. Lin CY, Lin CC, Hwang B, Chiang B. Serial changes of serum interleukin-6, interleukin-8, and tumor necrosis factor alpha among patients with Kawasaki disease. J Pediatr 1992; 121: 924-6.
15. Matsubara T, Katayama K, Matsuoka T, Fujiwara M, Koga M, Furukawa S. Decreased interferon-gamma (IFN-gamma)-producing T cells in patients with acute Kawasaki disease. Clin Exp Immunol 1999; 116: 554-7.
16. Terai M, Jibiki T, Harada A, *et al.* Dramatic decrease of circulating levels

of monocyte chemoattractant protein-1 in ' Kawasaki disease after gamma globulin treatment. J Leukoc Biol 1999; 65: 566-72.
17. Hirao J, Hibi S, Andoh T, Ichimura T. High levels of circulating interleukin-4 and interleukin-10 in Kawasaki disease. Int Arch Allergy Immunol 1997; 112: 152-6.
18. Noh GW, Lee WG, Lee W, Lee K. Effects of intravenous immunoglobulin on plasma interleukin-10 levels in Kawasaki disease. Immunol Lett 1998; 62: 19-24.
19. Matsubara T, Umezawa Y, Tsuru S, Motohashi T, Yabuta K, Furukawa S. Decrease in the concentrations of transforming growth factor-beta 1 in the sera of patients with Kawasaki disease. Scand J Rheumatol 1997; 26: 314-7.
20. Koga M, Ishihara T, Takahashi M, Umezawa Y, Furukawa S. Activation of peripheral blood monocytes and macro-phages in Kawasaski disease: ultra-structural and immunocytochemical in-vestigation. Pathol International 1998; 48: 512-7.
21. Okada T, Harada K, Okuni M. Serum HDL-cholesterol and lipoprotein fraction in Kawasaki disease (acute mucocutaneous lymph node syndrome). Jpn Circul J 1982; 46: 1039-44.
22. Van Hinsbergh VW. LDL cytotoxicity. The state of the art. Atherosclerosis 1984; 53: 113-8.
23. Tanaka N. Kawasaki disease (acute febrile infantile muco-cutaneous lymph node syndrome) in Japan: relationship with infantile periarteritis nodosa. Path Microbiol Basel 1975; 43: 204-18.