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

Yasufumi Shikishima, Yutaka Kawano, Hiroyuki Shirai, Nobuo Matsuura and Takeshi Noma

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.

Macrophage-colony stimulating factor is a cytokine that stimulates formation of macrophage colonies by functionally activating mature macrophages and monocytes. This cytokine also influences the lipid metabolism, attenuating the concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol. Although elevated levels of M-CSF in Kawasaki disease have been documented, 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.

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.

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)
Table 1  Patients with Kawasaki disease

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Age</th>
<th>Sex</th>
<th>γ-globulin therapy</th>
<th>Other therapy</th>
<th>Cardiac involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 months</td>
<td>male</td>
<td>550 mg/kg/day, 5d</td>
<td>Flurbiprofen</td>
<td>Mitral insufficiency</td>
</tr>
<tr>
<td>2</td>
<td>11 months</td>
<td>female</td>
<td>400 mg/kg/day, 5d</td>
<td>Aspirin</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>4 years 6 months</td>
<td>female</td>
<td>390 mg/kg/day, 5d</td>
<td>Flurbiprofen</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>1 year 1 month</td>
<td>female</td>
<td>420 mg/kg/day, 5d</td>
<td>Aspirin</td>
<td>Reduced EF¹</td>
</tr>
<tr>
<td>5</td>
<td>10 months</td>
<td>female</td>
<td>490 mg/kg/day, 5d</td>
<td>Flurbiprofen</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>5 months</td>
<td>male</td>
<td>400 mg/kg/day, 5d</td>
<td>Aspirin</td>
<td>None</td>
</tr>
</tbody>
</table>

¹:ejection fraction

Table 2  Disease controls

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Age</th>
<th>Sex</th>
<th>Cervical Symptoms</th>
<th>Hyperemia Symptoms</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fever</td>
<td>Eruption</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lymph node</td>
<td>Conjunctiva</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Eruption</td>
<td>Lips</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 year 5 months</td>
<td>male</td>
<td>+</td>
<td>-</td>
<td>Bacterial pneumonia</td>
</tr>
<tr>
<td>2</td>
<td>1 year 10 months</td>
<td>female</td>
<td>+</td>
<td>-</td>
<td>Mycoplasma pneumonia</td>
</tr>
<tr>
<td>3</td>
<td>3 years 1 month</td>
<td>female</td>
<td>+</td>
<td>-</td>
<td>Mycoplasma pneumonia</td>
</tr>
<tr>
<td>4</td>
<td>1 year 2 months</td>
<td>female</td>
<td>+</td>
<td>+</td>
<td>Mycoplasma pneumonia</td>
</tr>
<tr>
<td>5</td>
<td>11 months</td>
<td>male</td>
<td>+</td>
<td>-</td>
<td>Viral meningitis</td>
</tr>
<tr>
<td>6</td>
<td>1 year 1 month</td>
<td>male</td>
<td>+</td>
<td>+</td>
<td>Measles</td>
</tr>
</tbody>
</table>

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 (Kirkegaard & 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](image1.png)

** 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](image2.png)

** 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).

![Graph showing correlation between M-CSF and peripheral blood monocyte counts](image)

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

![Graph showing serum concentrations of total cholesterol](image)

**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.
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, 5,17,18 TNF-α, 9,10,11,12,13 granulocyte colony-stimulating fac-
Thus, an aberrant lipid metabolism metabolites may induce oxidative in KD may be ascribed to the reduction of serum HDL/2the reduction of HDL-cholesterol in KD.21 Because cytotoxicity from LDL has demonstrated an inverse relationship between M-CSF and total cholesterol and HDL-cholesterol in KD. 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.

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


