Elevated serum levels of macrophage colony-stimulating factor in patients with Kawasaki disease complicated by cardiac lesions

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Abstract

Objective
The main pathogenic characteristic of Kawasaki disease (KD) is the activation of mononuclear phagocytes. The cytokines produced by activated monocytes/macrophages elicit proinflammatory and prothrombotic responses in endothelial cells. Thus, we speculated that macrophage colony-stimulating factor (M-CSF), derived from monocytes/macrophages or vascular endothelial cells, might play an important role in the pathogenesis of the acute phase of KD. The aim of this study was to investigate the possible role of M-CSF in the pathogenesis of KD and to elucidate the relationship between serum M-CSF levels and clinical features and cardiac lesions.

Methods
Using ELISA, we serially assayed M-CSF and several cytokines, including interleukin-6, interleukin-8, tumor necrosis factor-alpha and interferon-gamma in the sera of 32 KD patients aged 2 months to 4 years.

Results
The serum M-CSF level during the first week of illness was significantly higher than during the second week or thereafter (first week, median 1710.0; second week, 1121.0; third week, 867.3; fourth week, 909.4 U/ml, p < 0.001) in our KD patients. Serum M-CSF levels during the first week of illness were also higher in patients with mitral and/or aortic valvular insufficiency than in patients without cardiac complications. Furthermore, serum M-CSF levels in patients with persistent coronary dilatation were higher than in those with no cardiac complications.

Conclusion
M-CSF plays a critical role in the pathogenesis of KD and can be used as an indicator for the risks of valvulitis and coronary arteritis.

Key words
Kawasaki disease, macrophage colony-stimulating factor, mitral valvular insufficiency, coronary artery lesion.

Introduction

Kawasaki disease (KD) is an acute systemic vasculitis in infants and young children, which is complicated by the development of myocarditis and coronary artery lesions (1-3). Activation of monocytes/macrophages plays a pivotal role in the development of vasculitis during KD (4-8). The elevated levels of proinflammatory cytokines, including tumor necrosis factor-alpha, interleukin-1, and interleukin-6, produced by the activated monocytes/macrophages could elicit proinflammatory and prothrombotic responses in endothelial cells during this disease process. Increased levels of tumor necrosis factor-alpha or interleukin-6 have been reported in those patients who develop coronary artery lesions (6,7). These cytokines can induce endothelial cell activation, which plays a role in the development of coronary artery lesions. The initial endothelial damage might be augmented by infiltrated monocytes/macrophages in the vascular lesions, which implies that a chemotactic cytokine recruits monocytes from the circulation (9-11). In fact the massive infiltration of monocytes/macrophages into the vascular lesions has been reported during the acute phase of KD (12).

Recently, we found high levels of serum macrophage colony-stimulating factor (M-CSF) and granulocyte colony-stimulating factor in patients in the acute phase of KD (13). M-CSF was originally purified from urine as a hematopoietic factor that stimulates the survival, proliferation, differentiation and several functions of mononuclear phagocytes (monocytes/macrophages) (14-16). The major species of M-CSF in the serum is an homodimeric secreted glycoprotein of 85 kDa17. Interferon-gamma, tumor necrosis factors alpha and beta, interleukin-1, interleukin-3, interleukin-4 and granulocyte-macrophage colony-stimulating factor induce M-CSF production by monocytes, endothelial cells, fibroblasts, T cells and polymorphonuclear leukocytes (16-19). M-CSF stimulates granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, and interleukin-6 production in vivo and in vitro (16,19). M-CSF has been shown to be involved in bone metabolism, pregnancy, cholesterol metabolism, and atherogenesis (20-24).

Recently, it was reported that M-CSF could play a role in the regulation of inflammatory and immune responses during inflammation and infection (19). During the inflammatory process, the early local production of M-CSF stimulates blood monocytes to migrate and to activate the local defenses. An early elevation in blood and tissue M-CSF concentrations is followed by an increase in activated macrophages with immunosuppressive activity.

The finding of elevated levels of serum M-CSF level in patients with KD supports functional evidence of monocyte/macrophage activation, and thus the magnitude and persistence of M-CSF secretion could be related to the development of myocarditis, valvulitis, and coronary arteritis in KD. In the present study, we report on the possible relationship between serum M-CSF levels and the severity of myocarditis, valvulitis, or coronary arteritis in KD. We also discuss the relationship between the serum levels of M-CSF and related cytokines, and clinical features such as cardiac lesions.

Materials and methods

Patients

Between September 1998 and January 2000, 32 patients at seven child-care or clinical centers in Japan were diagnosed with KD according to the criteria established in 1984 by the Kawasaki Disease Research Committee in Japan (25). The day of fever onset was recognized as the first day of illness. All patients were enrolled in the study within seven days of the fever onset. Informed consent was obtained from the patients’ parents.

Our treatment consisted of high-dose intravenous gamma globulin and aspirin. All 32 patients took aspirin orally at a dose of 30 mg/kg/day. Gamma globulin was administered to 27 patients at 400 mg/kg/day for 5 consecutive days, whereas 3 patients received 2000 mg/kg as a single infusion, and 2 patients received 400 mg/kg/day for 3 days. Treatment was started between the second and ninth day of illness in all pa-
tients. In 4 patients, additional intravenous gamma globulin was infused because fever persisted for 48 hours after the initial gamma globulin infusion or because echocardiograms suggested the progression of coronary dilatations.

We assessed the pathological changes in the coronary artery by two-dimensional echocardiography before the intravenous gamma globulin infusion and every week thereafter. Coronary artery dilatations > 3 mm in diameter or >1.5 times the adjacent vessel diameter were defined as aneurysmatic lesions. A transient coronary dilatation was a dilatation that had regressed by four weeks, and a persistent coronary dilatation was one that persisted for four weeks. The shortening fraction of the left ventricle, the function of the heart valves, and the absence or presence of pericardial effusion were evaluated by two-dimensional and color Doppler echocardiography.

Serum cytokine levels in 10 healthy children, 7 males and 3 females aged three months to five years (mean 32 ± 23 months), were studied as age-matched controls. Serum M-CSF levels in 6 vasculitis patients (2 patients with anaphylactoid purpura, 2 patients with systemic lupus erythematosus, and 1 patient with juvenile rheumatoid arthritis) in the acute phase were also studied as disease controls.

Methods
Blood samples for the cytokine assay and laboratory examination were collected serially within the first week after the onset of fever, and then once a week for the first month. Blood samples were taken on the first to seventh day (mean 4.5 ± 1.6; first week) of illness before treatment with intravenous gamma globulin or aspirin, between days 8 and 14 (mean 11.1 ± 1.7; second week), between days 15 and 21 (mean 17.5 ± 1.8; third week), and between days 22 and 33 (mean 24.3 ± 2.8; fourth week).

We routinely examined the blood cell counts and erythrocyte sedimentation rates (ESR), and the serum levels of C-reactive protein, electrolytes, aspartate aminotransferase, alanine aminotransferase, total protein, and albumin. The sera were stored frozen at -80°C until the cytokine assays were performed.

The M-CSF concentration was determined by ELISA as described previously (26). Briefly, the ELISA was based on the “dual antibody immunometric sandwich” principle using horse and rabbit polyclonal antibodies against human urinary colony-stimulating factor (CSF-HU). The minimal detectable level of hM-CSF was 10 U/ml. The average serum hM-CSF level of 20 normal adults was 540 ± 110 U/ml (range 300 to 800 U/ml). Interleukin-6, interleukin-8, tumor necrosis factor-alpha, interferon-gamma were also assayed by ELISA (Amersham).

Statistical analysis
The significance of the results was evaluated by Wilcoxon’s signed-rank test, the Mann-Whitney U test, or Spearman’s correlation coefficient. Statistical significance was defined as a p value less than 0.05.

Results
Clinical characteristics
The 32 patients ranged in age from 2 months to 4 years (mean 23 months; median 22 months); 18 of the patients were male. The duration of fever ranged from 2 to 23 days (mean 7.5 days; median 7.0 days). Three patients exhibited transient coronary artery dilatation and 3 patients were found to have persistent coronary artery dilatation. During the first week of illness 10 KD patients exhibited mitral and/or aortic valvular insufficiency, although the valvular insufficiency disappeared thereafter. Among the 10 valvulitis patients, 2 showed impaired left ventricular contractility, i.e. <0.28 of the left ventricle shortening fraction, and 2 patients had an apparent pericardial effusion. Only 2 patients exhibited both mitral valvular insufficiency and coronary artery dilatation. The age, duration of fever, and laboratory data were not significant higher in the patients with mitral and/or aortic valvular insufficiency or those with coronary artery lesions than in the patients with no cardiac complications (p > 0.05, Mann-Whitney U test).

Serial serum levels of M-CSF
As shown in Figure 1, during the first and second weeks, we observed a significant elevation of serum M-CSF levels in the KD patients compared to the normal age-matched controls (p < 0.05, Mann-Whitney U test). Serum M-CSF returned to baseline levels within 2 weeks after the administration of high-dose gamma globulin. No difference was observed in serum M-CSF levels between patients in the convalescent phase (third and fourth week of illness) and the normal age-matched controls (p > 0.05, Mann-Whitney U test).

Serial serum levels of M-CSF in patients with KD. Blood samples at the first week of illness obtained before intravenous gamma globulin infusion and aspirin use. The serum concentration of M-CSF was assayed by enzyme-linked immunosorbent assay. Serum concentrations are shown in KD patients (open circles) and in age-matched controls (closed circles); the bar represents the median.

* P < 0.05. ** P < 0.01; compared with age-matched control; # P < 0.001; compared with the first week of illness.

Fig. 1. Serial serum levels of M-CSF in patients with KD. Blood samples at the first week of illness obtained before intravenous gamma globulin infusion and aspirin use. The serum concentration of M-CSF was assayed by enzyme-linked immunosorbent assay. Serum concentrations are shown in KD patients (open circles) and in age-matched controls (closed circles); the bar represents the median.

* P < 0.05. ** P < 0.01; compared with age-matched control; # P < 0.001; compared with the first week of illness.
week were significantly higher than those in the second week or thereafter (p < 0.001, Wilcoxon’s signed-rank test). Serum M-CSF levels during the acute phase of KD were significantly higher than in the 6 vasculitis controls (median 1295.4 U/ml, range 892.1 - 2698.3 U/ml, p < 0.05, Mann-Whitney U test).

Serum M-CSF levels during the acute phase of KD with or without cardiac complications
As shown in Figure 2, during the first week of illness, serum M-CSF levels in patients with mitral and/or aortic valvular insufficiency were significantly higher than in those without (median 2829.8 versus median 1596.1 U/ml, p < 0.05, Mann-Whitney U test). During the first week of illness, serum M-CSF levels in all patients with coronary dilatation were not significantly higher than in patients with no cardiac complications (median 2004.3 U/ml versus median 1596.1 U/ml, p > 0.05, Mann-Whitney U test). Similarly, serum M-CSF levels in patients with transient coronary dilatation were not higher than in patients without cardiac complications (p > 0.05, Mann-Whitney U test). However, serum M-CSF levels in patients with persistent coronary dilatation were higher than in patients without cardiac complications (p < 0.05, Mann-Whitney U test).

Relationship of serum M-CSF levels to other cytokines and laboratory data
Serum M-CSF levels were not correlated with serum levels of interleukin-6, interleukin-8, tumor necrosis factor-alpha, and interferon-gamma during the first week of illness (p > 0.05, Spearman’s correlation coefficient). Similarly, serum M-CSF levels were not correlated with clinical indicators such as fever duration nor with the laboratory data such as blood cell counts, ESR, serum levels of C-reactive protein, electrolytes, aspartate aminotransferase, alanine aminotransferase, total protein and albumin (p > 0.05). The levels of serum M-CSF and other cytokine are shown in Table I.

Serum interleukin-6 levels during the first week were significantly higher than in the second week or thereafter (p < 0.05, Wilcoxon’s signed-rank test). Serum levels of interleukin-6 correlated with serum C-reactive protein levels in the acute phase (p < 0.05, Spearman’s correlation coefficient), but they did not correlate with platelet counts over the course of the illness (p > 0.05). Serum interleukin-8 levels were variable during the acute phase and thereafter, but did not differ significantly between the acute or subacute phases and the convalescent phase (p > 0.05, Wilcoxon’s signed-rank test). Serum interleukin-8 levels were not correlated with leukocyte or neutrophil counts (p > 0.05, Spearman’s correlation coefficient).

Table I. Serial serum levels of M-CSF, interleukin-6, interleukin-8, tumor necrosis factor-alpha, and interferon-gamma in patients with Kawasaki disease.

<table>
<thead>
<tr>
<th>Week of illness</th>
<th>M-CSF (U/ml)</th>
<th>Interleukin-6 (pg/ml)</th>
<th>Interleukin-8 (pg/ml)</th>
<th>TNF-alpha (pg/ml)</th>
<th>INF-gamma (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>median</td>
<td>range</td>
<td>median</td>
<td>range</td>
<td>median</td>
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<tr>
<td>1</td>
<td>1710.0</td>
<td>689.4 - 3896.0</td>
<td>27.3</td>
<td>0 - 283.8</td>
<td>13.9</td>
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<td>2</td>
<td>1121.1</td>
<td>634.6 - 3162.0</td>
<td>0</td>
<td>0 - 283.7</td>
<td>11.8</td>
</tr>
<tr>
<td>3</td>
<td>867.3</td>
<td>350.7 - 3318.5</td>
<td>0</td>
<td>0 - 2.7</td>
<td>7.9</td>
</tr>
<tr>
<td>4</td>
<td>909.4</td>
<td>334.7 - 1227.4</td>
<td>0</td>
<td>0 - 4.2</td>
<td>4.7</td>
</tr>
<tr>
<td>Control</td>
<td>811.3</td>
<td>532.4 - 1197.0</td>
<td>0</td>
<td>0 - 0</td>
<td>116.5</td>
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<td>0 - 12.57</td>
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| TNF: tumor necrosis factor; INF: interferon.
Serum levels of tumor necrosis factor-alpha and interferon-gamma in the acute phase were not significant higher than those in the convalescent phase (p > 0.05, Wilcoxon’s signed-rank test).

Discussion
In the present study we found elevated serum M-CSF levels during the acute phase of KD. The serum M-CSF levels were significantly higher in the patients with cardiac complications (i.e., mitral valvular insufficiency or persistent coronary dilatation) than in those without cardiac complications. This is the first report to describe the relationship between cardiac involvement in KD and a serum factor, M-CSF. Our data suggest that during the acute phase of KD, M-CSF might be a critical factor in the development of valvulitis and coronary arteritis, which are induced by the activation of monocytes/macrophages and the production of proinflammatory cytokines.

The major histopathological finding detected in autopsied specimens from KD patients is panvasculitis with endothelial necrosis, and the infiltration of mononuclear cells into the small and medium-sized blood vessels (12). Neutrophils are present in the very early stage, but rapidly give way to large mononuclear cells accompanied by lymphocytes and plasma cells. Cytokine-mediated cardiac dysfunction and pathogenesis in myocardial disorders found higher serum levels of interleukin-1 alpha, interleukin-1 beta, tumor necrosis factor-alpha, and M-CSF in acute myocarditis (28). Tumor necrosis factor-alpha protein is expressed in the myocardium in fatal myocarditis, and tumor necrosis factor-alpha is associated with myocardic damage in human myocarditis (28, 29). Tumor necrosis factor-alpha produces myocardial depression through a direct effect on calcium handling and the augmentation of nitric oxide synthetase expression (30).

In the present study, serum tumor necrosis factor-alpha did not increase concomitantly with serum M-CSF. Although the mechanism of myocarditis and valvulitis in KD is still unclear, M-CSF might play an important role in the pathogenesis of valvulitis in KD. An association between serum cytokine levels and the formation of coronary arterial lesions was reported previously for acute KD (31). Serum levels of interleukin-1, interleukin-2, soluble interleukin-2 receptor, interleukin-6, interleukin-8, tumor necrosis factor-alpha, and interferon-gamma in acute or subacute phases of KD were higher in patients with coronary artery lesions. Tumor necrosis factor-alpha was found to induce vascular injury in KD patients and serum levels of tumor necrosis factor-alpha were higher in the second week of illness than in the first week in patients treated only with aspirin (3, 6-10, 13, 31). Though serum tumor necrosis factor-alpha levels were not elevated during the course of the present study, tumor necrosis factor-alpha production was most likely already depressed during the acute phase after the injection of high-dose gamma globulin.

Interleukin-8 is a chemo-attractant factor that causes leukocyte infiltration (32). Pathological studies have shown that infiltration by neutrophils occurs only during the early phase of KD, and that mononuclear cells infiltrate thereafter (12). Other studies have found that during the first week of illness, KD patients with coronary artery lesions treated orally with aspirin had significantly higher serum interleukin-8 levels than patients without coronary artery lesions (31). However, in the present study serum levels did not reflect interleukin-8 activity. One possible explanation for this observation is that interleukin-8 may not be a major chemotactic factor in the acute phase of KD. Recent immunohistochemical studies on the cardiac tissues of patients with fatal KD indicated that monocyte chemoattractant protein-1, but not interleukin-8 or macrophage inflammatory protein-1 alpha, is localized at the extracellular matrix associated with mononuclear cellular infiltration (11). We propose that M-CSF may induce the synthesis of monocyte chemotactic protein 1 by endothelial cells, thereby enhancing the migration of monocytes into the subendothelial layer.

The beneficial effect of intravenous gamma globulin was observed in all patients in the present study. However, the efficacy of gamma globulin treatment in KD is not fully understood (3, 33, 34). One possible cause is the rapid down-regulation of the immune response. The binding of gamma globulin dimers to low affinity Fe-gamma receptors may result in the secretion of cytokines that down-regulate inflammatory responses, or in the down-regulation of the secretion of cytokines that cause inflammation (3,33,34). In the present study, after gamma globulin treatment serum M-CSF levels rapidly fell to baseline levels, serum interleukin-6 and C-reactive protein levels decreased, and the symptoms disappeared. The cytokines, including M-CSF, which elicit proinflammatory responses in endothelial cells may be reduced by high-
dose gamma globulin. Two patients whose serum M-CSF levels did not fall below 2000 U/ml after the injection of gamma globulin had a persistent fever and elevated C-reactive protein. Further study of the cases resistant to gamma globulin treatment and the indications for additional steroid therapy is required.

Acknowledgment
We thank Ms. Noriko Wakimoto and Mr. Munee Yamada (Biochemical Research Laboratory, Morinaga Milk Industry, Zama, Kanagawa, Japan) for their cooperation in this study.

References