Prognostic Impact of Vascular Leakage in Acute Kawasaki Disease

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Background—Increased microvascular permeability is an initial step of Kawasaki disease (KD). We reported that vascular endothelial growth factor (VEGF) might play a role in the vascular leakage of KD. In fatal KD, plasma leakage was extensively documented at VEGF-positive microvessels. Increases in vascular leakage cause hypoalbuminemia and noncardiogenic edema. However, the prognostic impact of vascular leakage in KD remains unclear.

Methods and Results—We compared 76 patients who became afebrile within 5 days after starting intravenous gamma globulin (IVGG) (2 g/kg over 5 days) (IVGG-responsive) with 27 patients who did not respond (IVGG-resistant). Baseline levels of serum VEGF and albumin were similar between the groups. After IVGG, VEGF levels increased (P<0.0001) and albumin levels decreased (P<0.00001) in both groups. However, the IVGG-resistant group had higher VEGF levels (P=0.029) and severe hypoalbuminemia (P<0.0001) compared with the IVGG-responsive group. Coronary aneurysms were documented in 12 patients from the IVGG-resistant group but not in the IVGG-responsive group. Then IVGG-resistant patients were divided into 2 subgroups according to the presence (n=12) or absence (n=15) of coronary aneurysms. There was no difference between subgroups in age, sex, laboratory data including albumin, and retreated doses of IVGG. However, body weight gain after IVGG was documented in patients who subsequently developed coronary aneurysms (P=0.003) but not in those who did not (P=0.967).

Conclusions—These results suggest that vascular leakage may be a key feature of KD pathophysiology. The present study may provide better insights into the pathogenesis and treatment of patients resistant to IVGG in acute KD. (Circulation. 2003;108:325-330.)

Key Words: plasma ■ vasculature ■ aneurysm ■ edema

Kawasaki disease (KD) is a systemic vasculitis. KD inflammation initially involves microvessels (capillaries, arterioles, and venules).1,2 During this initial process, increased microvascular permeability is an important event in KD vasculitis.1,3 We recently reported that vascular endothelial growth factor (VEGF) might play a role in the vascular leakage of KD.3,4 Elevated levels of circulating VEGF were observed when skin rash or edema of the hands and feet appeared in KD.3 Serum albumin levels were inversely related to serum VEGF levels.4 In fatal KD, plasma leakage was extensively documented and was associated with edema at VEGF-positive microvessels.4 Increases in vascular leakage cause hypoalbuminemia and noncardiogenic edema. However, studies on the prognostic impact of vascular leakage in KD are lacking.

Intravenous gamma globulin (IVGG) is clearly effective in the rapid resolution of KD inflammation.5,6 However, approximately 10% to 20% of patients had persistent or recurrent fevers after IVGG completion and are considered to have a higher risk of developing coronary aneurysms.7-10 The management of these IVGG-resistant patients is unestablished. It is unknown how much vascular leakage occurs in IVGG-resistant patients or those who develop coronary aneurysms despite IVGG treatment. We hypothesized that vascular leakage is important in KD pathophysiology. To test our hypothesis, we investigated serum albumin and VEGF levels and the degree of edema in IVGG-responsive and IVGG-resistant patients.

Methods

Definition of IVGG-Responsive and IVGG-Resistant

Patients from January 1998 through October 2002 at Chiba University were studied. Only patients who met the criteria for KD (displaying at least 5 major symptoms) were included. Excluded were patients who did not receive IVGG or those with a baseline echocardiogram indicating coronary abnormalities. A total of 103 patients, ages 2 to 98 months, were examined in this study. Of these 103 patients, 88 patients were initially treated at Chiba University. The remaining 15 patients were initially treated at Chiba University and were referred to our hospital for additional acute KD treatment because after IVGG they remained febrile (n=4) or developed...
coronary aneurysms (n=11). Medical records were available for all 15 referral patients.

All patients were initially treated with IVGG (400 mg/kg per d) for 5 consecutive days (2.0 g/kg total dose). In addition, they received oral aspirin (30 mg/kg per d) or an intravenous heparin infusion. Nine of 103 patients received intravenous dexamethasone (0.3 mg/kg per d for 3 consecutive days) along with initial IVGG in a multicenter trial to investigate effects of dexamethasone on KD outcome. Combined therapy did not change any inflammatory laboratory data, including albumin and VEGF, or coronary artery outcomes. Therefore, none of these patients with dexamethasone laboratory data, including albumin and VEGF, or coronary artery diseases such as small ventricular septal defects were studied as controls. Informed consent for VEGF measurements was obtained from the parents of all studied patients.

Treatment was performed in some selected patients with severe hypoalbuminemia at outside hospitals but not at Chiba University. Consequently, 6 of 15 referral patients had received albumin infusion along with initial IVGG.

An axillary temperature was taken, with fever defined as ≥37.5°C. Patients who became afebrile within 5 days of starting IVGG were classified as IVGG-responsive (76 patients). Patients who had persistent fever up to the sixth day of IVGG treatment were defined as IVGG-resistant (27 patients). IVGG-resistant patients were treated with additional IVGG (n=25) or corticosteroids (n=6). None received ulinastatin during the course of illness.

Body Weight
To monitor the degree of edema, we measured body weight on admission and every morning in all patients who were initially treated at Chiba University. Up to 13 kg of body weight was measured using a digital scale with sensitivity of 2 g (Atom Medical). In older patients with body weight greater than 13 kg, a different digital scale with sensitivity of 50 g (A&D) was used. All IV boards were preweighed, and the weight was noted on the board. All weights were without clothes and were obtained before any oral intake and then corrected by subtracting the weight of the preweighed IV board. In the other 15 patients who were initially treated at outside hospitals, body weight change before and the day after IVGG completion was determined from their medical records. In their hospitals, body weight was measured primarily in the same way, using their digital scales.

Measurement of VEGF and Albumin
VEGF levels were measured using a commercially available ELISA kit (R&D Systems), as previously reported. Serum samples were collected before the first IVGG dose and the following day after completion of the initial IVGG dose (2 g/kg). Serum albumin levels were also analyzed in the same samples. The correlation of VEGF and albumin levels was reported to be inversely related. VEGF levels from 6 patients who were treated at outside hospitals were not investigated because serum samples were unavailable. Serum VEGF levels in 20 patients, ages 2 to 85 months, who had noncyanotic heart diseases such as small ventricular septal defects were studied as control. Informed consent for VEGF measurements was obtained from the parents of all studied patients.

Statistics
All data are shown as mean±SD. The difference in the number of patients between groups was analyzed by Fisher’s exact possibility test. Values between groups were compared using the Mann-Whitney U test, and those before and after IVGG were compared with paired t tests. P<0.05 was considered significant.

Results
Comparison of Laboratory Data Between IVGG-Responsive and -Resistant Groups
We first compared baseline demographic and laboratory data between the IVGG-responsive and IVGG-resistant groups (Table 1). Age was not different (P=0.889) between the groups, but the male/female ratio was higher (P=0.005) in the IVGG-resistant group. Illness duration at IVGG initiation was 4.2±1.1 days in the IVGG-responsive group compared with 5.3±1.3 days in the IVGG-responsive group (P=0.00006). Baseline levels of VEGF (P=0.828) and albumin (P=0.633) were similar between the groups, although baseline levels of white blood cells (P=0.041), C-reactive protein (P=0.010), and GPT (P=0.012) were different between the groups (Table 1).

The IVGG-responsive patients had a more rapid resolution of inflammation compared with the IVGG-resistant patients. After IVGG, C-reactive protein decreased from 8.0±5.6 to 1.3±1.0 mg/dL (P<0.00001) in the IVGG-responsive patients and increased from 11.0±6.4 to 13.4±7.6 mg/dL (P=0.07) in the IVGG-resistant patients.

VEGF levels in control patients were 231±114 pg/mL. VEGF concentrations of the IVGG solution were under the level of sensitivity of this assay. After IVGG, serum VEGF levels increased from 748±451 to 1014±599 pg/mL (P<0.0001) in the IVGG-responsive group and from 586±389 to 1176±581 pg/mL (P<0.0001) in the IVGG-resistant group. After IVGG, serum albumin levels decreased

<table>
<thead>
<tr>
<th>Variable</th>
<th>IVGG-Responsive Group (n=76)</th>
<th>IVGG-Resistant Group (n=27)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of males</td>
<td>36</td>
<td>21</td>
<td>0.005</td>
</tr>
<tr>
<td>Age at onset, mo</td>
<td>Mean/median 31/30</td>
<td>33/25</td>
<td>0.889</td>
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<tr>
<td></td>
<td>SD 23</td>
<td>26</td>
<td></td>
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<tr>
<td></td>
<td>Range 2 to 82</td>
<td>3 to 98</td>
<td></td>
</tr>
<tr>
<td>Illness duration when IVGG started</td>
<td>Mean/median 5.3/5.0</td>
<td>4.2/4.0</td>
<td>0.00006</td>
</tr>
<tr>
<td></td>
<td>SD 1.3</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range 3 to 8</td>
<td>3 to 7</td>
<td></td>
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<tr>
<td>WBC counts/μL</td>
<td>Mean/median 15 379/14 650</td>
<td>13 655/12 700</td>
<td>0.041</td>
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<tr>
<td></td>
<td>SD 5117</td>
<td>5395</td>
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</tr>
<tr>
<td></td>
<td>Range 7200 to 33 000 5700 to 29 300</td>
<td></td>
<td></td>
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<tr>
<td>C-reactive protein, mg/dL</td>
<td>Mean/median 8.0/6.1</td>
<td>11.0/9.9</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>SD 5.6</td>
<td>6.4</td>
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<tr>
<td></td>
<td>Range 0.3 to 28.0</td>
<td>4.1 to 30.6</td>
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<td>GPT, IU/L</td>
<td>Mean/median 69/26</td>
<td>159/164</td>
<td>0.012</td>
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<tr>
<td></td>
<td>SD 105</td>
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</tr>
<tr>
<td></td>
<td>Range 6 to 464</td>
<td>9 to 567</td>
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<tr>
<td>Albumin, g/dL</td>
<td>Mean/median 3.93/4.0</td>
<td>3.86/4.0</td>
<td>0.633</td>
</tr>
<tr>
<td></td>
<td>SD 0.37</td>
<td>0.40</td>
<td></td>
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<tr>
<td></td>
<td>Range 2.9 to 4.9</td>
<td>3.1 to 4.5</td>
<td></td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>Mean/median 748/634</td>
<td>586/420</td>
<td>0.828</td>
</tr>
<tr>
<td>(n=75)</td>
<td>SD 451</td>
<td>389</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range 109 to 1932</td>
<td>214 to 1621</td>
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</table>
from 3.93±0.37 to 3.50±0.35 g/dL (P<0.00001) in the IVGG-responsive group and also decreased from 3.86±0.40 to 2.86±0.41 g/dL (P<0.00001) in the IVGG-resistant group (Figure 1). After IVGG, the IVGG-resistant group had higher VEGF levels (P=0.029) and lower albumin levels (P<0.00001) compared with the IVGG-responsive group. When 9 patients with dexamethasone were excluded from the analyses (all IVGG-responsive), we obtained similar statistical results (data not shown).

**Coronary Aneurysm**

None of the IVGG-responsive patients developed coronary dilation or aneurysms (>3 mm) after 2 weeks of illness. In contrast, 12 patients of the IVGG-resistant group developed coronary aneurysms after 2 weeks of illness. Seven of the 12 patients had giant coronary aneurysms with internal diameters greater than 8 mm. Two patients had middle-sized aneurysms (5 to 8 mm), and the remaining 3 patients had small aneurysms or dilated changes (3 to 4 mm).

**Laboratory Data and Additional Treatments in IVGG-Resistant Patients**

Next, we divided the 27 IVGG-resistant patients into 2 subgroups according to the presence (n=12) or absence (n=15) of coronary aneurysms. There was no significant difference between the subgroups with respect to age at KD onset, sex, baseline and posttreatment white blood cell counts (data not shown), C-reactive protein, GPT (data not shown), and albumin (Table 2). However, serum VEGF levels after IVGG were higher in the patients who subsequently developed coronary aneurysms than in those who did not develop coronary aneurysms (P=0.030).

Retreated IVGG doses and the number of patients treated with steroids after initial IVGG were similar between the subgroups (Table 2). However, albumin treatment (total dose=14.0±3.1 g over 2 to 4 days; range, 10 to 16 g) along with the initial IVGG was performed for 6 IVGG-resistant patients, all of whom subsequently developed coronary aneurysms (P=0.001). Of the 6 patients with albumin treatment, 4 had giant aneurysms and the remaining 2 had middle-sized aneurysms. Serum albumin levels were 3.7±0.5 g/dL before IVGG and 2.7±0.3 g/dL after IVGG in these 6 patients. In the other 21 patients without albumin treatment, serum albumin levels were 3.9±0.4 g/dL before IVGG and 2.9±0.4 g/dL after IVGG.

**Body Weight Change Before and After IVGG**

To evaluate the degree of edema, we investigated body weight change before and after IVGG. These data were available in all 76 IVGG-responsive patients and in 25 of 27 IVGG-resistant patients, including 5 patients who received albumin infusion. Increases in body weight after IVGG completion were found in 26 patients (34%) of the IVGG-responsive group and in 16 patients (64%) of the IVGG-resistant group (P=0.008 by Fischer’s exact possibility test).

Next, we divided the 25 IVGG-resistant patients with body weight data into 2 subgroups according to the presence (n=11) or absence (n=14) of coronary aneurysms. In the patients who developed coronary aneurysms (CAV), body weight before and after IVGG increased by 6% from 10.275±3.77 to 10.892±4.27 kg (P=0.003, Figure 2). Increase rate was 5.7% in 5 patients with albumin and 6.3% in 6 other patients without albumin. In the patients who had normal coronary arteries (NON-CAV), body weight changed from 13.515±4.48 to 13.506±4.60 kg (P=0.967, Figure 2).

**Outcome of IVGG Treatment Without Albumin Infusion at a Single Institution**

Finally, we studied outcomes for 88 KD patients who were initially treated at Chiba University. None of the 88 patients received albumin treatment. Seventy-six patients (87.4%) responded to IVGG and completely recovered without coronary artery involvement. Twelve patients (13.6%) were resistant to IVGG, 1 of whom (1.1% of the total) developed coronary aneurysms after 2 weeks of illness.

**Discussion**

This study demonstrates that vascular leakage may be a key feature of KD pathophysiology. IVGG-resistant patients were characterized by higher VEGF production and severe hypoalbuminemia compared with the IVGG-responsive group. Of these IVGG-resistant patients, body weight gain after IVGG was evident in patients who subsequently developed coronary aneurysms, suggesting the presence of increased vascular leakage in these patients.

KD is characterized by systemic vasculitis with tissue edema and microvascular sequestration of inflammatory cells, which are all seen in many organs of fatal KD. Tissue edema is already seen during the initial phase of KD. For example, edematous changes of extremities are a striking feature of KD and occur within the first 5 days of illness. In older children, cervical lymph node swelling may develop before fever onset. Endothelial gap formation and subendothelial edema are documented in the skin, suggesting the presence of vascular permeability. However, there has been less focus on the impact of vascular leakage. If vascular leakage is a key feature of acute KD, the degree of edema may serve as an important clinical symptom to predict KD outcome. For this reason, we investigated body weight gain to assess edema severity and found that patients who developed coronary aneurysms were more likely to gain weight after initial IVGG.
Pathological increases in vascular leakage are features of diabetic retinopathies, rheumatoid arthritis, and acute respiratory distress syndrome. VEGF and its receptors are overexpressed in these diseases. VEGF is known to be a potent vascular permeability factor, which induces fenestrae in venular and capillary endothelia. We suspect that VEGF may be one of the mediators of microvascular permeability of KD. Recent studies reveal that edema and plasma leakage are found in VEGF-positive microvessels including vasa vasorum. Previous investigators have demonstrated that the
presence of edema and elevation of serum VEGF levels might be risk factors for coronary artery outcome. Insufficient oxygen supply from the vasa vasorum of coronary arteries may cause local hypoxia in the media of these arteries and play a role in progression to coronary aneurysms of KD. On the other hand, VEGF induces NO production from vascular endothelial cells by increasing both NO synthase enzyme expression and activity in vitro. It was recently reported that NO produced by the interaction of VEGF and its receptor mediates VEGF-induced vascular permeability. Previous investigators have demonstrated a possible role of NO in acute KD.

IVGG treatment is clearly effective in reducing the incidence of coronary aneurysms from 15% to 25% to <5%. In the United States, IVGG is given in a single 2-g/kg dose. In Japan, 2 g/kg is often administered over 4 to 5 days because the medication with a single-dose regimen is not government-approved. Although the mechanisms of IVGG action remain unknown, the single 2-g/kg dose seems to accelerate the laboratory indexes of acute inflammation rapidly normalized, including serum albumin and C-reactive protein levels. Nonetheless, the percentage of patients resistant to IVGG seems to be similar in both regimens. Significant differences in the coronary outcome have not been shown in these 2 approaches.

Effects of IVGG on VEGF production have not been investigated in acute KD illness. Inflammatory cytokines are elevated in the circulation of patients during the acute stage of KD illness, including interleukin-6, interleukin-8, and monocyte chemoattractant protein-1. These elevated levels decreased after IVGG treatment when patients responded to IVGG. Similarly, acute-phase reactants rapidly decreased or normalized after IVGG treatment in IVGG responders. In this study, however, elevated levels of VEGF were still persistent despite IVGG treatment in both IVGG-resistant and IVGG-responsive patients. In the present study, we used a commercially available ELISA kit (R&D Systems), which measures free (active) VEGF as previously shown by others. We confirmed that the IVGG solution did not contain VEGF protein. It is unknown whether increased production of VEGF is a primary or secondary event, but elevation of VEGF levels was associated with a reduction of serum albumin levels. Although future studies are necessary to clarify whether VEGF really contributes to vascular leakage of KD, effects of IVGG on inhibition of vascular leakage is likely to be limited in patients resistant to IVGG.

Albumin is the most abundant plasma protein, and it has the lowest molecular weight among the plasma proteins. It is the principal solute responsible for colloid osmotic pressure, which produces an effective osmotic gradient across capillary walls. In Japan, low albumin level is used as one of the risk factors in selection of candidates for IVGG treatment of KD. For this reason, in Japan, albumin infusion is sometimes performed to correct hypoalbuminemia in the early stage of acute KD, especially when edema is extensive, but effects of albumin infusion on KD outcome are untested. The role of colloid replacement remains controversial in cases of noncardiogenic edema with hypoproteinememia. Some investigators reported that diuresis with albumin infusion led to normalization of serum protein concentration and hemodynamics in patients with acute lung injury but had no effects on mortality. Others reported that albumin infusion aggravated pulmonary edema in rats with endotoxemia and in patients with sepsis-induced respiratory distress syndrome. In this study, all patients who received albumin infusion along with initial IVGG developed body weight gain after IVGG and later developed larger coronary aneurysms. Although selection bias related to the use of albumin cannot be ruled out, we speculate that albumin administration may accelerate interstitial edema attributable to additional albumin leakage at microvessels in KD cases, which may increase the risk of developing larger coronary aneurysms.

In conclusion, this study suggests that vascular leakage may be a key feature of KD pathophysiology. Our data may suggest the clinical importance of monitoring body weight to evaluate the severity of vascular leakage. The present study may provide better insights into the pathogenesis and treatment of patients resistant to IVGG in acute KD.

Acknowledgments

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References
