Impairment of Angiogenic Activity in the Serum From Patients With Coronary Aneurysms Due to Kawasaki Disease

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Background The inflammatory mediators play an important role in the progression of coronary vasculitis in Kawasaki disease (KD), but effects of KD serum including inflammatory mediators on endothelial cells remain unknown. We hypothesized that serum activity to stimulate in vitro human umbilical vein endothelial cells (HUVEC) tube formation might be impaired in KD.

Methods and Results Serum from patients with coronary aneurysms was less active in stimulating HUVEC tube formation than serum from patients without coronary aneurysms or febrile controls. In patients with coronary aneurysms, the reduction in the serum angiogenic activity was documented already before KD treatment (p=0.03 vs healthy controls, p=0.08 vs febrile controls) and enhanced after intravenous immune globulin plus aspirin (p<0.001 vs healthy controls, p=0.002 vs febrile controls); both drugs did not affect the assay studied. This reduction was greater in patients who later developed giant aneurysms >8 mm compared with those who developed small to moderate aneurysms (p=0.01). The reduced serum angiogenic activity was partly caused by the reduction in the serum activity of stimulating HUVEC proliferation.

Conclusions Serum activity to stimulate HUVEC tube formation was impaired in KD patients who later developed larger coronary aneurysms, which may be associated with the severity of vascular injury. (Circ J 2007; 71: 1052–1059)

Key Words: Angiogenesis; Endothelial cell; Inflammation; Kawasaki disease

Kawasaki disease (KD) is a systemic vasculitis predominantly affecting young children. Intravenous immune globulin (IVIG) is effective in the rapid resolution of the inflammation of KD. However, approximately 3–5% of patients develop coronary aneurysms. Once giant coronary aneurysms develop, they might thrombose, thus leading to sudden death. Mortality rates because of coronary artery thrombosis in patients with KD are especially high in the first 2 months of the disease.1–3 Even after this critical period, patients with giant coronary aneurysms have a greater risk of myocardial infarction in the first year of illness.4

Endothelial cells of aneurysms were injured and denuded in patients who died in the acute or subacute stage of illness.5 The vascular endothelia are critical components within the circulatory system. The coordinated activation of endothelial cells by inflammatory cytokines and angiogenic growth factors promote re-endothelialization of injured vessels.6 These inflammatory mediators appear to play an important role in the progression and repair of coronary vasculitis in KD, although the precise mechanisms have not been elucidated. Various proinflammatory cytokines are produced in the sera of patients with acute KD, including tumor necrosis factor-α, interleukin-6, interleukin-8, and monocyte chemoattractant protein-1.7–9 Serum levels of vascular endothelial growth factors are also elevated in the acute illness.10–12 Less is known about the effects of KD serum including various inflammatory mediators on vascular endothelial cells. Previous investigators examined effects of serum angiogenic activity on vascular endothelial cells by using cultured human umbilical vein endothelial cells (HUVEC) in patients with Wegener’s granulomatosis, Takayasu disease and giant-cell arteritis, and found that serum angiogenic activities increased in the patients with vasculitides.13–14 We hypothesized that serum activity to stimulate in vitro HUVEC tube formation might be impaired in KD patients, especially in those who later develop coronary aneurysms. Therefore, we examined the KD serum angiogenic activity to stimulate cultured HUVEC.

Methods

Reagents

Salicylic acid, dexamethasone and warfarin were purchased from Sigma, and aspirin (acetylsalicylic acid) was purchased from Wako. Heparin sodium was purchased from Aventis Pharma and prednisolone sodium succinate from Shionogi. Immune globulin was kindly provided by Teijin Pharma.

Treatment Protocol and Sample Selection

One hundred and fifty-one patients from February 1999 through to November 2004 were admitted to Chiba University. Excluded were patients with a baseline echocardiogram indicating coronary abnormalities, those who did not
receive IVIG, or those administered with dexamethasone along with an initial IVIG in a multicenter trial to investigate the effects of dexamethasone on KD outcome. Thus, a total of 121 patients were included for serum sample selection (Fig 1).

As shown in Fig 1, 101 patients initially treated at Chiba University were treated with an initial IVIG for 1–5 days (2.0g/kg total dose). They also received oral aspirin (ASA, 30mg·kg⁻¹·day⁻¹) (n=58) or an intravenous heparin infusion (10units·kg⁻¹·h⁻¹) (n=43) because the effects of IVIG plus heparin on KD outcome were examined in 43 patients. Five patients (3 in IVIG+ASA and 2 in IVIG+heparin) developed coronary aneurysms later on.

Among patients who responded to initial IVIG, 20 patients (30.1±23.1 months old at KD onset) (10 with IVIG plus aspirin and 10 with IVIG plus heparin) were randomly selected (Fig 1). In these patients, a total of 79 samples were available for the study. They were serially obtained at pre-IVIG (n=20, day 5.2±1.3 [mean±standard deviation (SD)], range, 3 to 8]), post-initial IVIG (n=20, day 9.9±1.5 [8 to 12]), 1 month (n=20, day 38.1±7.6 [20 to 53]), and 1 year after KD onset (n=19).

In an aneurysmal group, a total of 17 patients (44.6±33.1 months old at KD onset) with coronary aneurysms were studied, including 12 patients initially treated at an outside hospital (Fig 1). Eight patients had small to medium coronary aneurysms (3.7–7.0 mm) and the remaining 9 had giant aneurysms (>8.0 mm). Eleven of the 17 patients received steroids after the completion of an initial IVIG. After resolution of acute KD inflammation, all 17 patients with coronary aneurysms were treated long-term with a low dose of aspirin (3–10mg·kg⁻¹·day⁻¹) to prevent thrombotic cardiovascular events. Seven of the 17 patients with coronary aneurysms received long-term warfarin (0.08–0.3mg·kg⁻¹·day⁻¹) combined with a low dose of aspirin. In the 17 patients with coronary aneurysms, a total of 60 samples were available for study. Serum samples were obtained at pre-IVIG (n=13, day 5.1±1.4 [3 to 7]), post-initial IVIG (n=17, day 9.6±3.2 [5 to 17]), 1 month (n=17, day 29.9±5.8 [22 to 41]), and 1 year (n=13) after the onset of KD.

For study reference, serum samples were collected from 10 healthy young children (46.6±29.6 months) and 8 febrile patients (45.9±31.3 months) with acute infection caused by group A streptococci and adeno or influenza virus. In addition, the serum from 2 patients with active Takayasu arteritis was assayed. All aliquot samples were frozen until use. The study was approved by the institutional review committee of Chiba University School of Medicine. Informed consent for the study was obtained from the parents both of patients and controls.

**Endothelial Cell Culture**

HUVEC were obtained from Cambrex and were cultured in endothelial cell basal medium (EBM-2, Cambrex) maintained at 37°C in 5% CO₂ and supplemented with 5% fetal bovine serum, penicillin/streptomycin, and endothelial cell growth supplement (SingleQuots, Cambrex). The cells were used in experiments between passage number 2 and 4.

**Endothelial Cell Tube Formation Assay**

Growth factor-reduced Matrigel (BD Biosciences) was placed in the well of a pre-chilled 48-well cell culture plate (Asahi Technoglass) and incubated at 37°C for 30 min to allow polymerization. HUVEC, at concentrations of 3×10⁴ per well, were plated into the growth factor-reduced Matrigel-coated wells, incubated at 37°C in 5% CO₂ for 18 h in the presence of 5% serum from either a patient or a control, and photographed using an inverted phase contrast photomicroscope at a magnification of 40× (Leica Microsystems). Photos of 4 different areas of each sample were taken and printed, which covered 80% of each well. The tube length was manually measured in each photo and total tube length was calculated. A mean of the total tube length at the 4 different areas was determined and included in the final data. The serum from the same healthy adult volunteer was always used as an internal control in the assay, and the percent increase or decrease in tube formation relative to the internal control was calculated for each sample. Each sample was examined in triplicate and the mean and SD was calculated.

**Endothelial Cell Proliferation Assay**

This endothelial cell proliferation was estimated using Calcein AM, as described previously, with minor modifica-
Statistics

HUVEC tube length and proliferation of internal control serum were defined as 100%. HUVEC tube length and proliferation for each sample are shown as a percent relative to the internal control serum (healthy adult volunteer). All parameters were presented as mean ± SD. A Mann-Whitney U-test was used to compare the data of serum angiogenic activities between KD patients and controls. For data from KD patients before and during steroid therapy, a paired t-test was applied. P values of <0.05 were considered significant.

Results

Experimental Conditions for In Vitro Angiogenesis

The conditions of the in vitro angiogenesis assay were adjusted so that HUVEC were capable of forming capillary-like tube structures in the well coated by growth factor-reduced Matrigel in the presence of healthy human serum, but failed in the absence of healthy human serum (Fig 2).

Serum Angiogenic Activity in KD Patients Who Had Normal Coronaries

Laboratory data in the sera used in this study are shown in Table 1. Serum from healthy or febrile controls promoted HUVEC tube formation and its activity were 95±6% and 93±13% of internal controls, respectively (Table 2). In 20 patients who had normal coronaries, the serum angiogenic activity at pre-IVIG was normal (89±22% of internal control, p=0.78 vs healthy control, p=0.63 vs febrile control). It

Table 1 Laboratory Data of KD Patients and Febrile Controls

<table>
<thead>
<tr>
<th>Study group</th>
<th>Illness after onset (Day, n=)</th>
<th>WBC (×10^3/μl)</th>
<th>Platelet (×10^3/μl)</th>
<th>CRP (mg/dl)</th>
<th>ALT (IU/L)</th>
<th>Albumin (g/dl)</th>
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<tbody>
<tr>
<td>Patients with normal coronaries</td>
<td>Pre-IVIG (Day 5.2±1.3, n=20)</td>
<td>15.2±5.3</td>
<td>37.8±9.4</td>
<td>9.3±5.5</td>
<td>75.3±8.2</td>
<td>4.0±0.4</td>
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<td></td>
<td>Post-initial IVIG (Day 9.9±1.5, n=20)</td>
<td>9.1±2.7</td>
<td>58.5±19.7</td>
<td>1.5±0.8</td>
<td>34.8±29.0</td>
<td>3.5±0.3</td>
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<td></td>
<td>1 month (Day 38.1±7.6, n=20)</td>
<td>9.5±3.2</td>
<td>43.9±14.2</td>
<td>0.1±0.2</td>
<td>15.6±8.6</td>
<td>4.5±0.3</td>
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<td></td>
<td>1 year (n=19)</td>
<td>8.2±2.0</td>
<td>37.9±9.2</td>
<td>0.1±0.3</td>
<td>12.9±2.8</td>
<td>4.4±0.2</td>
</tr>
<tr>
<td>Patients with coronary aneurysms</td>
<td>Pre-IVIG (Day 5.1±1.4, n=13)</td>
<td>14.9±4.9</td>
<td>32.3±13.5</td>
<td>14.1±8.4**</td>
<td>94.5±96.2</td>
<td>3.7±0.5</td>
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<tr>
<td></td>
<td>Post-initial IVIG (Day 9.6±3.2, n=17)</td>
<td>20.0±10.6**</td>
<td>46.1±23.0*</td>
<td>15.2±9.0***</td>
<td>32.1±18.8</td>
<td>2.9±0.6**</td>
</tr>
<tr>
<td></td>
<td>1 month (Day 29.9±5.8, n=17)</td>
<td>12.4±7.8</td>
<td>64.0±28.2*</td>
<td>0.8±1.1***</td>
<td>19.8±12.0</td>
<td>4.0±0.3**</td>
</tr>
<tr>
<td></td>
<td>1 year (n=13)</td>
<td>8.5±3.3</td>
<td>35.8±5.7</td>
<td>0.1±0.3</td>
<td>11.7±2.7</td>
<td>4.5±0.3</td>
</tr>
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<td>Febrile controls</td>
<td></td>
<td>14.6±6.4</td>
<td>30.8±8.7</td>
<td>3.1±3.6</td>
<td>15.0±10.3</td>
<td>4.5±0.3</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 vs patients with normal coronaries, Mann-Whitney U-test.

KD, Kawasaki disease; WBC, white blood cells; CRP, C-reactive protein; ALT, alanine transferase; IVIG, intravenous immune globulin.
reduced to 79±20% at post-initial IVIG (p=0.02 vs healthy control, p=0.05 vs febrile control) and to 81±17% at 1 month (p=0.02 vs healthy control, p=0.09 vs febrile control). At 1 year after KD onset, the serum angiogenic activity of KD patients with normal coronaries (89±18% of internal control) was similar to that of healthy controls (p=0.32) or febrile controls (p=0.98) (Table 2).

Serum Angiogenic Activity in KD Patients Who Later Developed Coronary Aneurysms

Serum angiogenic activity at the pre-IVIG stage in patients who later developed coronary aneurysms was significantly reduced compared with healthy controls, but this was not the case for febrile controls and patients with normal coronaries (73±14% of internal control, p=0.03 vs healthy control, p=0.08 vs febrile control, p=0.45 vs patients with normal coronaries) (Table 2; Fig 2). This reduction in serum angiogenic activity in this subgroup was further enhanced at post-initial IVIG (63±24%, p=0.001 vs healthy control, p=0.002 vs febrile control, p=0.06 vs patients with normal coronaries), and at 1 month after KD onset (50±24%, p<0.001 vs healthy control, febrile control, and patients with normal coronaries). This impairment was non-existent at 1 year after disease onset (65±23%, p=0.05 vs healthy control, p=0.09 vs febrile control, p=0.10 vs patients with normal coronaries).

Proliferating Activity of HUVEC in the Sera After IVIG is Different Between Patients With and Without Coronary Aneurysms

Capillary-like tube formation requires several biological activities, such as endothelial cell proliferation, cell migration, and cell-to-cell interaction. To investigate mechanisms of impairment of HUVEC tube formation promoted by sera from patients who later developed coronary aneurysms, we additionally examined serum activity to proliferate HUVEC. The serum activity of healthy and febrile controls was 95±8% and 120±18% of the internal controls, respectively (Table 3). When treated with KD serum at pre-IVIG, the serum activity was 129±17% of the internal controls in patients with normal coronaries, and it was 113±13% (p=0.08 vs patients with normal coronaries) in patients who later developed coronary aneurysms (Table 3). The external addition of KD serum at post-initial IVIG enhanced the difference in the serum activity between patients with and without coronary aneurysms. Thus, serum from patients who later developed coronary aneurysms was less active in stimulating HUVEC proliferation compared with serum from patients with normal coronaries (89±14% vs 108±14%, p=0.008) or febrile controls (p=0.005).

Relationship of Serum Angiogenic Activity to the Coronary Aneurysm Severity

To investigate the effects of vascular injury severity on

Table 2  In Vitro Angiogenesis in Human Umbilical Vein Endothelial Cells Promoted by Sera From Patients and Controls of Comparable Ages

<table>
<thead>
<tr>
<th>Study group</th>
<th>Illness after onset</th>
<th>Tube length</th>
<th>p values</th>
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<tr>
<td></td>
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<td>% of internal control</td>
<td>% of febrile control</td>
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<tr>
<td>Healthy control (n=10)</td>
<td></td>
<td>95±6</td>
<td></td>
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<tr>
<td>Febrile control (n=8)</td>
<td></td>
<td>93±13</td>
<td>100</td>
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<tr>
<td>KD with normal coronaries</td>
<td>Pre-IVIG (n=20)</td>
<td>89±22</td>
<td>96</td>
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<tr>
<td>(n=20)</td>
<td>Post initial IVIG (n=20)</td>
<td>79±20</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>1 month (n=20)</td>
<td>81±17</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>1 year (n=19)</td>
<td>89±18</td>
<td>96</td>
</tr>
<tr>
<td>KD with coronary aneurysms</td>
<td>Pre-IVIG (n=13)</td>
<td>73±14</td>
<td>79</td>
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<tr>
<td>(n=17)</td>
<td>Post initial IVIG (n=17)</td>
<td>63±24</td>
<td>68</td>
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<tr>
<td></td>
<td>1 month (n=17)</td>
<td>50±24</td>
<td>54</td>
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<tr>
<td></td>
<td>1 year (n=13)</td>
<td>65±23</td>
<td>70</td>
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</table>

Mann-Whitney U-test.
Abbreviations see in Table 1.

Table 3  In Vitro Proliferation in Human Umbilical Vein Endothelial Cells Promoted by Sera From Patients and Controls of Comparable Ages

<table>
<thead>
<tr>
<th>Study group</th>
<th>Illness after onset</th>
<th>Proliferation % of internal control</th>
<th>p values</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>vs healthy control</td>
<td>vs febrile control</td>
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<tr>
<td>Healthy control (n=10)</td>
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<td>95±8</td>
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<tr>
<td>KD with normal coronaries</td>
<td>Pre-IVIG (n=10)</td>
<td>129±17</td>
<td>&lt;0.001</td>
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<tr>
<td>(n=10)</td>
<td>Post initial IVIG (n=10)</td>
<td>108±14</td>
<td>0.006</td>
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<td>KD with coronary aneurysms</td>
<td>Pre-IVIG (n=8)</td>
<td>113±13</td>
<td>0.004</td>
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<tr>
<td>(n=8)</td>
<td>Post initial IVIG (n=8)</td>
<td>89±14</td>
<td>0.09</td>
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Mann-Whitney U-test.
Abbreviations see in Table 1.
serum angiogenic activity, we compared the serum from 9 patients who later developed giant coronary aneurysms with 8 patients who later developed small to moderate coronary aneurysms. To exclude the effects of steroid on serum activity, we studied serum samples at pre- and post-initial IVIG, both of which did not involve steroid use. There were no differences in the demographic data including aspirin and initial IVIG doses between these groups (data not shown). Pre-IVIG serum angiogenic activity to stimulate HUVEC tube formation (68±13% of internal control, p=0.42 vs patients with normal coronaries) in 7 patients who later developed giant coronary aneurysms was lower than in 6 patients (79±14%, p=0.71 vs patients with normal coronaries) who later developed small to medium aneurysms, however, the difference was not significant (p=0.47 vs patients with small to moderate aneurysms) (Fig 3). Furthermore, the difference in the serum angiogenic activity was statistically significant between the groups when samples at post initial IVIG were used. Thus, the serum angiogenic activity to stimulate HUVEC tube formation was 49±25% in internal controls (p=0.007, **p=0.01, Mann-Whitney U-test).

Fig 3. Comparison of serum angiogenic activity between patients with small to medium coronary aneurysms and those with giant coronary aneurysms. Numbers represent the sample number studied. Serum angiogenic activity to stimulate human umbilical vein endothelial cells (HUVEC) tube formation is significantly different after initial intravenous immune globulin (IVIG). CAL, coronary artery lesions; NS, not significant. Each bar shows the mean±SD. *p=0.007, **p=0.01, Mann-Whitney U-test.

Fig 4. (A) Two treatment regimens for acute Kawasaki disease (KD). Arrows indicate the time-points for obtaining serum samples. (B) Serum angiogenic activity was not significantly different between the 2 treatment regimens in the first year of illness. Numbers represent the sample number studied. Each bar shows the mean±SD. IVIG, intravenous immune globulin; NS, not significant.
to medium aneurysms (p=0.01) (Fig 3).

Effects of Aspirin Treatment on Serum Angiogenic Activity
Aspirin has an anti-angiogenic activity whereby it blocks prostaglandin synthesis in endothelial cells.17 To determine the effects of aspirin on serum angiogenic activity, we compared 10 patients receiving IVIG plus aspirin with 10 patients receiving IVIG plus intravenous heparin infusion without aspirin (Fig 4A). All 20 patients had normal coronaries throughout the illness. There were no differences in the baseline demographics and total IVIG doses between these 2 treatment regimens (data not shown). The serum angiogenic activity was similar between the 2 treatment regimens up to 1 year after KD onset (Fig 4B).

We also tested the effects of the exogenous addition of acetylsalicylic or salicylic acid at concentrations of 0–1,000 μmol/L on HUVEC tube formation promoted by healthy control serum and found that HUVEC tube formation was significantly inhibited when treated with higher doses over 1,000 μmol/L (data not shown). Finally, we tested the serum from an adult healthy volunteer taking oral aspirin at a dose of 200 mg daily for 1 week. The serum angiogenic activity in this volunteer was 92% of the internal control at baseline, 99% at 30 min after intake, and 115% at 4 h after intake. Serum concentrations of salicylic acid were 4 μg/ml at 30 min after intake and 10 μg/ml (almost 3.6 μmol/L) at 4 h after intake.

Effects of Steroid Treatment on Serum Angiogenic Activity
Steroids can inhibit angiogenesis18,19 and so we tested the effects of the exogenous addition of prednisolone sodium succinate or dexamethasone at concentrations of 0–100 μmol/L on in vitro HUVEC tube formation promoted by healthy control serum and found that HUVEC tube formation was significantly inhibited by the external addition of these drugs at concentrations over 10 μmol/L (data not shown). Steroids at a concentration of 10 μmol/L correspond to a clinical dose of almost 1 mg/kg.

Furthermore, we studied the effects of steroid treatment on serum angiogenic activity using 9 paired samples before (9.2±1.6 day of illness) and during (29.8±7.2 day of illness) steroid therapy (Fig 5A). The angiogenic activity in the sera before steroid therapy was 62±29% of the internal control, and it was 50±29% during steroid therapy (p=0.007) (Fig 5B).

Effects of Other Drugs Used in KD Patients
We tested the effects of IVIG (0–1,000 μg/ml), warfarin (0–10 μg/ml) and heparin sodium (0–10 unit/ml) on serum angiogenic activity. These drugs did not affect in vitro HUVEC tube formation promoted by healthy human serum (data not shown).

Discussion
In the present study, we found that in vitro HUVEC tube formation was less in the presence of serum from KD patients who later develop coronary aneurysms, whereas it was same in the presence of serum from KD patients who later had normal coronaries, compared to healthy controls. This result was enhanced after initial IVIG and at 1 month of illness in patients who were resistant to IVIG and developed larger coronary aneurysms. Even 1 year after the KD onset, the serum angiogenic activity in KD patients with coronary aneurysm still tended to be lower compared with healthy controls (p=0.05). Pathological angiogenesis is believed to be mediated by various pro-angiogenic and anti-angiogenic factors in the human body. It has been previously reported that an imbalance exists in the production between pro-angiogenic and anti-angiogenic factors in KD patients with coronary artery lesions (CAL).20 In KD patients, some molecules, such as vascular endothelial growth factor and endostatin, are suggested to influence CAL involvement.11,20 Our data provide more evidence for
this hypothesis. KD patients who sustain the state of the imbalance might have a greater risk of CAL involvement.

To investigate the mechanisms of reduced angiogenic activity in the serum from KD patients with coronary aneurysms, we studied the inhibitory effects of drugs on serum angiogenic activity. Aspirin is widely used for patients with coronary aneurysms to prevent thromboembolic events. It is well known that aspirin causes irreversible inhibition of platelet cyclooxygenase-1, resulting in the inhibition of thromboxane A2 formation. However, aspirin has an anti-angiogenic activity in that it blocks prostaglandin synthesis in endothelial cells through the inhibition of cyclooxygenase2 and therefore, even small doses of aspirin might cause impairment of in vitro HUVEC tube formation promoted by human serum. To explore this possibility, we tested the difference in the serum angiogenic activity between KD patients treated with IVIG plus aspirin and those treated with IVIG and heparin. To exclude the effects of coronary aneurysm formation, we investigated samples from patients without coronary aneurysms. As a result, we found that HUVEC tube formation promoted by serum was not different throughout the illness between these 2 groups. Furthermore, additional experiments with the exogenous addition of acetylsalicylic (aspirin) or salicylic acid at various doses confirmed that a low concentration of acetylsalicylic or salicylic acid did not show significant inhibitory effects on HUVEC tube formation promoted by human serum. We measured the serum concentration of salicylic acid, which has a longer plasma half-life than aspirin (15 min half-life), in a healthy volunteer taking a low dose of aspirin for 1 week, and found that a low serum concentration of salicylic acid in this volunteer did not influence the ability of serum to promote in vitro HUVEC tube formation. Furthermore, in patients with coronary aneurysms, impaired serum angiogenic activity was already detected before IVIG plus aspirin treatment. With these results taken together, it was unlikely that the aspirin used for KD treatment was the main cause of impairment of the in vitro HUVEC tube formation in the presence of serum from KD patients who developed coronary aneurysms.

In contrast, it is known that dexamethasone or glucocorticoids also inhibit angiogenesis18,19. In addition, dexamethasone has inhibitory effects on E-selectin, VCAM-1 and ICAM-1 expressions in HUVEC. Indeed, we found that the external addition of steroids at concentrations over 10 μmol/L inhibited in vitro HUVEC tube formation promoted by healthy control serum. In addition, serum samples during steroid therapy reduced serum activity to stimulate HUVEC tube formation compared with samples taken prior to steroid therapy. Taken together, it was suggested that steroid therapy might accelerate the impairment of in vitro HUVEC tube formation promoted by human serum. As long-term changes in coronary artery aneurysms is related to acute therapeutic regimens21 further investigation into the effects of steroids on endothelial repair might be necessary.

Interestingly, serum angiogenic activity seems to be different among different vascular inflammatory diseases. Previous investigators reported increased serum angiogenic activity using in vitro HUVEC tube formation in patients with Wegener’s granulomatosis, Takayasu disease and giant-cell arteritis. Indeed, using the similar assay we used here, we confirmed that 2 patients with acute Takayasu disease had increased serum angiogenic activity (unpublished data). In contrast, the present study documented the impairment of serum angiogenic activity in KD patients with coronary aneurysms. The mechanisms responsible for the differences in the serum angiogenic activity between KD and other types of vasculitis are not clear, but it is possible that age might play a role. For example, giant-cell arteritis preferentially affects individuals over the age of 50 years, whereas KD patients are usually less than 5 years old when they are affected. This age difference might influence the magnitude of the production of proinflammatory cytokines associated with vascular injury.

In conclusion, serum activity to stimulate in vitro HUVEC tube formation was impaired in KD patients who later develop coronary aneurysms. Although further investigations are necessary to determine the mechanisms, angiogenic activity in the serum from KD patients might be associated with the severity of vascular injury.

References