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Neutrophils and Mononuclear Cells Express Vascular Endothelial Growth Factor in Acute Kawasaki Disease: Its Possible Role in Progression of Coronary Artery Lesions

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ABSTRACT

Kawasaki disease (KD) is a syndrome of systemic vasculitis of unknown etiology that is complicated by coronary artery lesions (CAL), leading occasionally to cardiac ischemic sequelae. To examine whether vascular endothelial growth factor (VEGF) is responsible for CAL in KD, we determined serum VEGF levels by ELISA and peripheral blood mononuclear cell (PBMC) and neutrophil VEGF expression by immunoblot analysis. Significantly increased levels of VEGF were demonstrated in acute KD as well as in other vasculitis syndromes ($p < 0.0001$). In the 10 KD patients with CAL, serum VEGF levels were maximal approximately 2 wk postonset when CAL generally develops and were significantly higher than in 20 patients without CAL (mean, 474 and 241 pg/mL, respectively; $p = 0.00015$). During the same period, immunoblot analysis revealed maximal VEGF expression in PBMC, corresponding to serum VEGF levels in most patients and being particularly marked in patients with CAL ($p < 0.01$). Neutrophils expressed VEGF only in the early stage of acute KD and declined rapidly in the majority of KD patients regardless of the presence of CAL, showing a strikingly different expression pattern than that for PBMC. Predominant VEGF expression by PBMC was also demonstrated in patients with other vasculitis syndromes and only faintly in normal controls. The results suggest that VEGF is generated dynamically in KD, presumably reflecting its disease activity. Neutrophil-derived VEGF may play a role in regulating early vascular responses, whereas PBMC-derived VEGF may contribute to later vascular injury and remodeling. **(*Pediatr Res* 49: 74–80, 2001)**

Abbreviations

VEGF, vascular endothelial growth factor
KD, Kawasaki disease
CAL, coronary artery lesion
PBMC, peripheral blood mononuclear cell

KD is an acute febrile illness of childhood that is characterized by clinical, biochemical, and histopathologic features of systemic vasculitis preferentially affecting coronary arteries (1). Although KD is usually a self-limited disease, serious CAL occur in 10 to 15% of affected children (2, 3). Since the decline of rheumatic fever, KD has become the primary cause of acquired heart disease in Japan and the United States. The etiologic nature of KD and the pathogenesis of CAL, however, largely remain to be understood. Previous histopathologic studies revealed that early coronary vascular lesions are infiltrated by a large number of mononuclear cells such as macrophages and lymphoid cells, leading to the assumption that these cells play a key role in the progression of CAL (4, 5). In acute KD, the migration and proliferation of endothelial cells have been demonstrated to be markedly enhanced by cytokines (6, 7). Thus, we may speculate that certain kinds of blood leukocytes actively generate some cytokines that enhance the proliferation and migration of endothelial cells in acute KD and serve as a trigger for vasculitis in this disease.

VEGF has been identified as a cytokine that regulates differentiation, proliferation, migration, and survival of cells in the microvascular endothelium (8, 9). Furthermore, VEGF has been shown to enhance vascular permeability and to modulate thrombogenicity. VEGF is expressed by a variety of cell types (10–13) including aortic smooth muscle cells (10), macrophages (11), and myocytes (12). In addition, lymphocytes (14), neutrophils (15, 16), and platelets (17) have recently been shown to express VEGF. In this article, we demonstrate that

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neutrophils as well as PBMC actively express VEGF in acute KD and are presumably involved in pathogenesis of vasculitis and the progression of CAL in this disease.

METHODS

Patients and blood samples. We studied the serum levels of VEGF in 160 children (aged 5 d to 14 y) whose conditions were classified into five groups: acute KD (n = 49), convalescent KD (n = 30), febrile illness (n = 28), other vasculitis syndromes (n = 15), and normal controls (n = 38) (Table 1). The acute and convalescent KD groups were further designated as those with or without CAL. In the convalescent group, the onset of KD had occurred at least 1 y before this study (mean, 3.7 y; range, 1 to 13 y). In the acute KD group, serum levels of VEGF were evaluated before the initiation of treatment (range, 4 to 7 d; mean, 5.2 d after onset). Febrile illnesses were viral infection (n = 15) and bacterial infection (n = 13). Other vasculitis syndromes included Henoch-Schölein purpura (n = 9), juvenile rheumatoid arthritis (n = 1), systemic lupus erythematosus (n = 3), Takayasu disease (n = 1), and mixed connective tissue disease (n = 1).

Changes in serum levels of VEGF over time were measured in 30 of the 49 patients with acute KD. In addition, we examined whether VEGF might be produced by blood leukocytes in these patients. For this purpose, immunoblot analysis of VEGF expression over time in PBMC and neutrophils was carried out in seven patients with CAL and seven patients without CAL, in comparison with such immunoblot analysis in three patients with other vasculitis syndromes or bacterial infection. In addition, to examine which of the PBMC subpopulations, lymphocytes or monocytes, is responsible for VEGF expression, flow cytometric analysis was performed in seven patients with CAL and seven normal controls. Blood samples were obtained before treatment and at 2 and 4 wk after the onset of KD. Blood samples were also obtained 2 mo after the onset from patients with CAL. Fresh heparinized venous blood was separated into neutrophils and mononuclear cells for immunoblot analysis as described below, and serum was stored at −80° until VEGF analysis by ELISA.

All patients and normal control individuals were seen at the University Hospital or one of seven affiliated hospitals. Blood samples were obtained between March 1995 and December 1998. All KD patients fulfilled the diagnostic criteria established by the KD Research Committee (18) and were treated with i.v. gamma globulin at 200 or 400 mg/kg for 5 d as well as oral aspirin (30 $mg \cdot kg^{-1} \cdot d^{-1}$). Two-dimensional echocardiography was performed before treatment with i.v. gamma globulin and at 2 wk, 4 wk, and 2 mo after the onset of KD. Time of disease onset was defined as the day on which fever appeared. A coronary artery with a diameter of 3 mm or more (4 mm if the subject was over the age of 5 y) was defined as abnormal according to the diagnostic criteria of the Research Committee on KD (19). Parental informed consent was obtained for each child enrolled in this study. The study was approved by the Research Ethics Committee of Toyama Medical and Pharmaceutical University Hospital.

VEGF measurement. The measurement of VEGF in the serum was performed by using an ELISA kit for human VEGF (Immuno-Biologic Laboratories Co. Ltd., Fujioka, Japan) according to the manufacturer-recommended procedures (20).

Immunoblot analysis of VEGF protein. Heparinized venous blood was separated into neutrophils and mononuclear cells by dextran sedimentation and Ficoll-Hypaque gradient centrifugation as previously described (21). Immunoblot analysis of VEGF expression in each cell population was performed as previously described (22). Briefly, 1 million cells were lysed with 10 μL lysis buffer (1% Triton-X 100, 10 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, 5 mmol/L EDTA, 2 mmol/L phenylmethylsulfonyl fluoride, 20 mmol/L *e*-amino-*n*-caproic acid, 20 mmol/L iodoacetamide, 0.01% soybean trypsin inhibitor, and 10 μL/mL aprotinin) for 40 min on ice and centrifuged for 10 min at 15,000 × g. The supernatants were mixed with equal volumes of SDS sample buffer and boiled for 3 min. The samples were size-fractionated on a 10% SDS-polyacrylamide gel and electroblotted on nitrocellulose filters. The blots were blocked in 5% skim milk in PBS (pH 7.4) for 1 h, incubated with rabbit polyclonal anti-VEGF (clone 147) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA,

Table 1. *Patient characteristics and serum VEGF levels according to study groups*

Groups	n	Age (y)	CRP (mg/dL)	WBC ($\times 10^3/mm^3$)	VEGF (pg/mL)	p value*
Acute KD	49	1.9 ± 0.2	9.2 ± 0.7	13.7 ± 0.9	281 ± 18	<0.0001
with CAL	14	2.5 ± 0.5	10.8 ± 1.7	16.0 ± 2.6	282 ± 37	<0.0001
without CAL	35	1.6 ± 0.2	8.9 ± 0.8	12.9 ± 0.7	280 ± 22	<0.0001
Convalescent KD	30	4.8 ± 0.7			52 ± 10	ns
with CAL	8	7.1 ± 1.7			26 ± 13	ns
without CAL	22	3.9 ± 6.6			61 ± 13	ns
Febrile illnesses	28	1.8 ± 0.3	4.7 ± 0.9	12.7 ± 0.9	111 ± 17	ns
Bacterial infection	13	1.9 ± 0.3	7.4 ± 0.8	14.3 ± 1.4	105 ± 22	ns
Viral infection	15	2.3 ± 0.7	2.3 ± 1.1	11.0 ± 1.0	112 ± 24	ns
Vasculitis syndromes	15	11.1 ± 1.6			233 ± 26	<0.0001
Normal controls	38	4.5 ± 0.7			74 ± 13	
Neonates	7	0.04 ± 0.02			193 ± 37	
Infants and children	31	5.5 ± 0.8			47 ± 7	

CRP indicates C-reactive protein; WBC, white blood cells.

* Difference in comparison with normal controls. Data are mean ± SEM.

U.S.A.) at 0.2 μg/mL or mouse monoclonal anti-β-actin antibody (Sigma Chemical Co. Chemical Co, St. Louis, MO, U.S.A.) at 1 μg/mL for 1 h, and subsequently incubated with 1:4000 dilution of peroxidase-labeled goat anti-rabbit IgG antibody or peroxidase-labeled goat anti-mouse IgG antibody (Biosource International, Camarillo, CA, U.S.A.), respectively, for 1 h. Between incubations, the blots were rinsed five times for 10 min each in PBS plus 0.05% Tween 20. Blots were developed using an enhanced chemiluminescence (ECL) Western blotting detection system (Amersham International plc, Buckinghamshire, UK). Prestained molecular weight markers (rainbow-colored protein; Amersham International plc) were used as standards for molecular size.

Densitometric quantitation of Western blots was performed with Densitograph software (Densitograph 4.0, Atto, Japan) and an imaging densitometer (Multiscan 20sf3, Sony, Japan). The density of VEGF bands was corrected relative to that of the β-actin band in each sample; the ratio of the VEGF value to that of β-actin was defined as the corrected VEGF (c-VEGF) value.

Flow cytometric assay. Two-color immunofluorescence analysis of VEGF expression in each subpopulation of mononuclear cells was performed as previously described (22). In brief, to discriminate between monocytes and lymphocytes, mononuclear cells were stained with phycoerythrin-labeled CD14 MoAb (IgG2a; Dako Japan Kyoto). Fixed permeabilized cells were reacted with rabbit polyclonal anti-VEGF Ab (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, U.S.A.) or control IgG1 MoAb (Dako Japan) for 20 min on ice, washed, and then incubated with a 1:2000 dilution FITC-conjugated goat anti-rabbit IgG antibody (Zymed Laboratories, San Francisco, CA, U.S.A.) for 20 min. The stained cells were analyzed on a Cytoron Absolute flow cytometer (Orth-Clinical Diagnostics, Tokyo, Japan). To quantify the data, DMFI was defined as differences of the mean fluorescence intensity between control antibody and anti-VEGF antibody.

Statistics. Results are summarized as mean ± SEM. Analysis of the differences between groups of patients was accomplished using ANOVA followed by the Dunnett multiple comparison test. Differences were considered significant at a p value < 0.05.

RESULTS

Serum VEGF levels. Serum VEGF levels in patients with acute KD were significantly higher than levels in normal controls ($p < 0.0001$) and in children with febrile illnesses ($p < 0.0001$) (Table 1). The elevation in serum VEGF levels was not seen in the convalescent phase of KD. There were no significant differences in serum VEGF levels in KD with or without CAL in the pretreatment acute stage or convalescent phases. Other vasculitis syndromes also demonstrated elevated levels of serum VEGF. Although they were included as normal controls in this study, neonates, unlike other children, displayed a moderate elevation in serum VEGF levels, indicating active physiologic angiogenesis during this period. Until 1 mo of age, serum VEGF levels decreased rapidly and were constant throughout childhood.

Table 2 shows a comparison of serum VEGF levels and clinical laboratory data over time in KD patients with ($n = 10$) and without ($n = 20$) CAL. Although serum VEGF levels before treatment were similar in patients with and without CAL, patients with CAL demonstrated higher serum VEGF levels than those without CAL 2 and 4 wk after onset ($p = 0.0015$ and $p < 0.0001$, respectively). There were no significant differences between these groups with respect to age, C-reactive protein, white blood cells, or duration of fever. The diameter of the coronary arteries in KD patients with CAL was observed to be maximal at 10 to 16 d (mean, 13.9 ± 1.1 d) after onset. Four of the 10 patients with CAL developed coronary aneurysms that were verified by selective coronary angiography in the convalescent phase.

VEGF expression in neutrophils and PBMC. As shown in Figure 1*A*, immunoblot analysis of neutrophils as well as PBMC before treatment detected isoforms of VEGF (30–46 kD) in two patients with acute KD and showed a slightly

Table 2. *Clinical laboratory data in acute KD with and without CAL*

	Acute KD with CAL	Acute KD without CAL	p value*
No. of patients	10	20	—
Age in y	2.5 ± 0.6	1.7 ± 0.3	ns
Max. CRP, mg/dL (mean ± SEM)	11.5 ± 1.2	9.1 ± 1.0	ns
Max. WBC, $\times 10^3$/mm^3 (mean ± SEM)	14.8 ± 0.6	13.0 ± 1.0	ns
Duration of fever, d (mean ± SEM)	7.5 ± 0.6	6.9 ± 0.4	ns
Max. diameter of coronary artery	4.3 ± 0.4	2.5 ± 0.1	
mm (mean ± SEM) (range)	(3.0–7.0)	(1.0–2.9)	
VEGF, pg/mL (mean ± SEM)			
pretreatment	321 ± 45	313 ± 35	ns
(mean illness d, range)	(5, 4–7)	(5, 3–7)	(ns)
2 wk postonset	474 ± 71	241 ± 28	0.0015
(mean illness d, range)	(12, 11–18)	(14, 10–19)	(ns)
4 wk postonset	297 ± 40	113 ± 9	<0.0001
(mean illness d, range)	(28, 22–36)	(28, 21–38)	(ns)
2 mo postonset	92 ± 12		
(mean illness d, range)	(56, 54–61)		

Values in parenthesis for VEGF indicate the days of blood sampling postonset of fever.
* Difference between groups.

dominant expression in neutrophils similar to that of the patient with bacterial infection. In contrast, VEGF expression in other vasculitis syndromes was more dominant in PBMC than in neutrophils. VEGF isoforms identifiable by immunoblot were also seen in PBMC from normal controls although at a lower level than that observed in acute KD. VEGF expression was negligible in neutrophils from normal controls (Fig. 1*B*).

Evaluation of VEGF expression over time identified different expression patterns in neutrophils and PBMC; neutrophils expressed VEGF isoforms only in the early stage of acute KD, whereas PBMC predominantly expressed VEGF isoforms at 2 wk after onset, continuing through 4 wk after onset (Fig. 1*B*). Figure 1*B* shows a representative immunoblot analysis of cellular VEGF expression throughout the clinical course of KD. The patient was a 3-y-old boy who developed multiple coronary aneurysms in the right and left coronary arteries approximately 2 wk after onset. His serum VEGF level was markedly elevated before treatment and peaked 2 wk after the onset despite the use of i.v. gamma globulin. VEGF levels gradually decreased with time and reached the normal range approximately 2 mo postonset. Immunoblot analysis clearly showed that neutrophils strongly expressed VEGF only from the acute phase to 2 wk after onset, whereas intense VEGF expression by PBMC was demonstrable beyond 4 wk.

Figure 2 depicts the changes in VEGF expression in neutrophils and PBMC over time in patients with and without CAL. A rapid decline in VEGF expression in neutrophils over time was seen in the majority of KD patients regardless of the presence of CAL. In contrast, most patients exhibited maximal VEGF expression in PBMC at 2 wk after onset, and these levels remained high beyond 4 wk after onset. In patients with CAL, VEGF expression by PBMC was exaggerated at 2 wk after onset, by which time most of the CAL had developed, and was maintained at higher levels than in patients without CAL (c-VEGF, 1.65 ± 0.09 *versus* 0.95 ± 0.11; $p < 0.01$). There were no significant differences in the pretreatment PBMC VEGF expression pattern between patients with and without CAL.

In the study using Western blot analysis, we could not evaluate whether both monocytes and lymphocytes may express VEGF in acute KD. However, the additional study using flow cytometric assay clearly showed that monocytes predominantly express VEGF rather than lymphocytes in seven patients with CAL during the subacute stage of KD (DMFI for monocytes and lymphocytes were 0.85 ± 0.36 and 0.35 ± 0.18, respectively; $p < 0.01$). In normal controls, monocytes and lymphocytes express VEGF faintly (DMFI for monocytes and lymphocytes were 0.15 ± 0.13 and 0.11 ± 0.10, respectively) (Fig. 3).

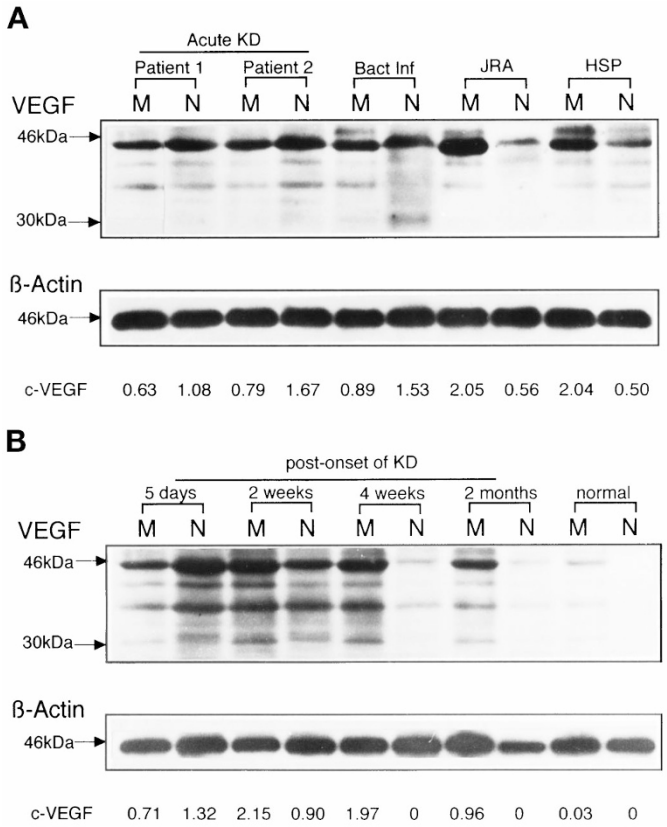

Figure 1. (*A*) Western blot analysis of VEGF isoforms (30–46 kD) before treatment in two patients with acute KD, in a patient with bacterial infection, and in patients with other vasculitis syndromes. Patient 1 has acute KD without CAL, and patient 2 has acute KD with CAL. *Bact Inf* indicates bacterial infection; *JRA*, juvenile rheumatoid arthritis; *HSP*, Henoch-Schönlein purpura; *M*, mononuclear cells; *N*, neutrophils. *C-VEGF* indicates the value of VEGF density corrected by that of β-actin. (*B*) Representative Western blot showing change in VEGF isoforms (30–46 kD) over time in a patient with CAL and in a normal control.

DISCUSSION

Previous studies have shown that various inflammatory cytokines such as IL-6, IL-8, and tumor necrosis factor-α (TNF-α) are elevated in the sera of patients with acute KD (23, 24). However, elevated serum levels of these cytokines simply indicate the intense inflammatory response in KD and do not seem to reflect the pathogenic nature of the vascular lesions particular to KD. Our study demonstrates that serum VEGF levels are greatly elevated in acute KD and may remain above normal values until the subacute stage when inflammatory

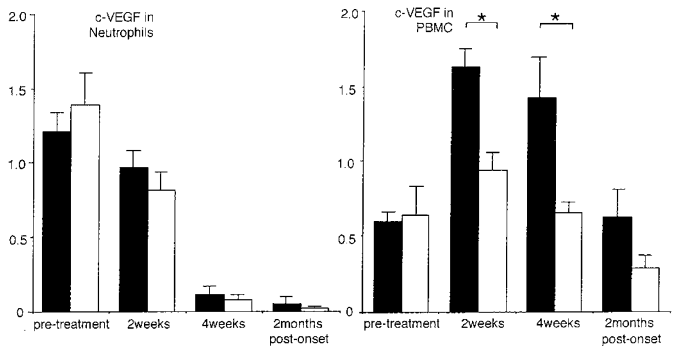

Figure 2. Comparison of the change over time in Western blot analysis of VEGF in neutrophils and in PBMC between patients with and without CAL. *Closed bars* show c-VEGF values (mean ± SE) in patients with CAL ($n = 7$); *open bars* show values in patients without CAL ($n = 7$). The density of VEGF bands was corrected relative to that of the β-actin band in each sample; the ratio of the VEGF value to that of β-actin was defined as the corrected c-VEGF value. $*p < 0.01$.

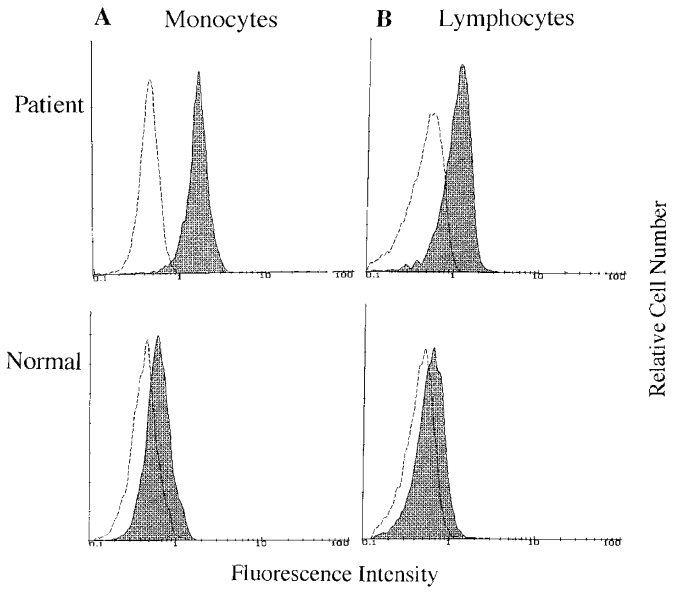

Figure 3. Flow cytometric analysis of intracellular VEGF expression in monocytes and lymphocytes from a patient with CAL at 2 wk postonset and from a normal control. The *dotted lines* and *solid lines* indicate the staining with control antibody and anti-VEGF antibody, respectively. Monocytes predominantly express VEGF rather than lymphocytes in a patient with CAL during the subacute stage of KD (DMFI for monocytes and lymphocytes were 0.78 and 0.41, respectively, in this patient). In normal control, monocytes and lymphocytes express VEGF faintly (DMFI for monocytes and lymphocytes were 0.17 and 0.14, respectively). Five thousand cells were evaluated in each gated population.

responses are clinically attenuated. In addition, KD patients who develop CAL may exhibit a prolonged and marked elevation in serum VEGF throughout the 2 to 4 wk after onset, during which most CAL are identified. It has been observed that certain serum components in acute KD can enhance proliferation and migration of endothelial cells (6, 7). VEGF is a novel cytokine with multiple actions including proliferation and migration mainly on endothelial cells and has been shown to be inducible in various cells by several inflammatory cytokines such as IL-6, IL-1β, transforming growth factor-β (TGF-β), and platelet-derived growth factor-β (PDGF-β) (25, 26). Thus, it is likely that VEGF is actively released into the blood pool of KD patients as a function of the disease.

It has been reported that activation of monocytes/macrophages plays a central role in the development of vasculitis in acute KD (27, 28). A previous histopathologic study demonstrated the infiltration of large numbers of mononuclear cells such as lymphocytes and macrophages into the vascular tissues of KD, implying that monocytes are recruited from the circulation in response to chemotactic stimuli (29). We demonstrated strong expression of VEGF in PBMC, especially in monocytes, until the subacute stages of the disease, corresponding to changes in serum VEGF levels. This tendency was especially marked in KD patients with CAL. Therefore, VEGF derived from mononuclear cells may play a pathogenic role in the development of CAL or vascular remodeling in response to injury associated with acute KD. In KD, it is thought that activated PBMC may be recruited to sites of the damaged endothelium and participate actively in the inflammatory responses of the vascular walls by locally producing VEGF as an endothelial cell-specific mitogen. VEGF has been described as an endothelial cell-specific mitogen. However, recent studies have documented that the VEGF receptor flt-1 is expressed in human monocytes as well, and VEGF induces the activation and migration of human myocytes *via* this receptor (30–32). Thus, monocyte-derived VEGF may induce chemotaxis of monocytes/macrophages in both the inflammatory and remodeling processes and may act in an autocrine or paracrine fashion in the coronary arterial walls of patients with acute KD. We could not perform pathologic analysis in this study, but recent immunohistochemical study clearly showed the prominent expression of VEGF on mononuclear cells in acute KD coronary artery tissue (personal communication, Suzuki *et al.*) and in the newly formed microvessels within the intima as well even in the late phase of KD (33). Our data of VEGF expression on PBMC appear to reflect such pathologic changes in KD coronary artery tissue.

In the present study, the intense expression of VEGF in PBMC corresponding to increased serum VEGF levels was also observed in other vasculitis syndromes despite a heterogenous group of diseases with different etiologies and underlying pathogenetic mechanisms. These vasculitis syndromes, including rheumatoid arthritis, are characterized pathologically by mononuclear cell infiltration. VEGF is reported to modulate endothelial function and angiogenesis in rheumatoid arthritis (34, 35). Therefore, increased serum levels of VEGF as well as VEGF expression by PBMC may reflect disease activity in such vasculitis syndromes.

Elevated serum or plasma levels of VEGF in acute KD have recently been reported by other investigators (36–38). It appears that serum VEGF levels before treatment could not predict the progression of CAL in acute KD in our study or in previous studies. Interestingly, in pretreatment acute KD, VEGF is predominantly expressed in neutrophils. VEGF expression in neutrophils was restricted to the early phase of acute KD and was detected in the early phase of bacterial infection as well but was negligible in other vasculitis syndromes and in normal controls. VEGF expression in neutrophils may reflect their activation in the acute phase of KD or enhanced neutrophilic function as reported previously (39). In acute KD, stimulation of and degenerative change in neutrophils similar to those observed in bacterial infection have also been reported (40). Neutrophil-derived VEGF may play an early restricted role in regulating vascular responses in acute inflammatory processes and may trigger vasculitis but may not have a direct effect on the progression of CAL.

It has been proposed that the interaction of endothelial cells and blood leukocytes plays a critical role in the development of vasculitis in KD (6, 7). Cytokines and antiendothelial cell antibodies may damage coronary vessel walls in acute KD by enhancing the migration of endothelial cells, and i.v. gamma globulin may prevent the progression of CAL by suppressing this endothelial cell migration (6, 7). VEGF possesses multipotent actions including inducing migration and proliferation of endothelial cells, enhancing vascular permeability, and modulating thrombogenicity, all of which have been documented experimentally *in vivo* and *in vitro* (8–13). Like other cyto-

kines, VEGF may promote coronary vascular damage by inducing the migration of endothelial cells and may play a critical role in the progression of CAL in acute KD. However, VEGF may have opposing effects on endothelial cells (33, 41, 42). A previous pathophysiologic study showed that VEGF may play some role in the progression of human coronary atherosclerosis as well as in the recanalization processes in obstructive coronary artery disease (41). Recent immunohistochemical study clearly showed the prominent expression of VEGF on coronary artery tissue of remodeling process in the late phase of KD (33). Another study reported that VEGF administration exacerbates neointimal thickening after vascular injury in dogs (42).

We did not use Western blot to assess VEGF secretion by platelets because of the fragility of platelets and the difficulty in performing a Western blot with these cells. A recent *in vitro* study showed constitutive production and thrombin-induced release of VEGF by human megakaryocytes and platelets (17). VEGF delivered to sites of vascular injury by activated platelets may contribute to the proliferation of endothelial cells and may initiate angiogenesis. Marked increase in platelet counts and activation of platelets are common findings in the subacute stage of KD. Therefore, we cannot neglect the possible importance of megakaryocyte lineage as one of the major sources of VEGF secretion in acute KD. Further study is needed to elucidate this issue.

In conclusion, the serum level of VEGF, a fraction of which is potentially generated from blood leukocytes, is elevated in KD, presumably reflecting its disease activity. Neutrophil-derived VEGF may play an early role in regulating vascular responses in acute inflammatory processes, whereas PBMC-derived VEGF may contribute to the vascular injury and remodeling observed in vasculitic changes. Interestingly, an angiogenesis inhibitor, AGM-1470 derived from *Aspergillus fumigatus*, has recently been reported to suppress the development of coronary arteritis in an animal model of KD (43). The modulation of VEGF may serve as a therapeutic strategy for the treatment of vasculitis syndromes including KD.

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