Jpn. J. Cancer Res. 87, 1143–1152, November 1996

Involvement of Vascular Endothelial Growth Factor in Kaposi's Sarcoma Associated with Acquired Immunodeficiency Syndrome

Shinsaku Sakurada,[1,4] Tetsuji Kato,[1] Kohichi Mashiba,[1] Shigeo Mori[2] and Takashi Okamoto[1,3]

[1]*Department of Molecular Genetics, Nagoya City University Medical School, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467 and* [2]*Department of Pathology, Institute of Medical Science, University of Tokyo, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108*

To examine the role of vascular endothelial growth factor (VEGF) in the development of edema associated with Kaposi's sarcoma (KS) in acquired immunodeficiency syndrome (AIDS), we exploited animal model systems to detect the activity that induces vascular hyper-permeability (VHP) using cultured AIDS-KS spindle cells. Cultured AIDS-KS spindle cells and conditioned medium (AIDS-KS-CM) that had been semi-purified through a heparin affinity column were tested for the ability to induce VHP in animals. The AIDS-KS spindle cells and AIDS-KS-CM induced VHP that was histamine-independent. The VHP-inducing activity was detected in the 0.5 *M* NaCl fraction from the heparin affinity column and was blocked by anti-VEGF neutralizing antibody. In addition, the production of VEGF was demonstrated in fresh AIDS-KS tissue as well as in cultured AIDS-KS cells, while control cells were negative for VEGF production. From these observations, we concluded that AIDS-KS cells produce a factor(s) that promotes VHP, and this factor could be VEGF.

Key words: Kaposi's sarcoma — Vascular endothelial growth factor — Acquired immunodeficiency syndrome — Edema

Tumor development often involves characteristic changes in blood vessels such as proliferation (neo-vascularization or angiogenesis) and vascular hyper-permeability (VHP).[1–3] Among the tumors with which vascular changes are associated, Kaposi's sarcoma (KS) is distinct, in that it is considered to originate from vascular endothelial or vascular smooth muscle cells[4–8] and it is associated with abundant angiogenesis and severe edema. Edematous lesions associated with KS have been described many times since the initial description by Kaposi himself in1872.[9] Severe edematous lesions in AIDS-KS sometimes cause life-threatening complications such as pulmonary edema, massive pleural effusion, and refractory protein-losing enteropathy, as well as swollen and painful extremities.[10–13]

Since the identification of factors from KS that induce angiogenesis,[14–16] it has been postulated that tumor-associated edema may be mediated by soluble factor(s) released from the tumor cells. Among such factors known to be produced from KS cells, VEGF (vascular endothelial growth factor) is unique in that it has potent angiogenic activity and it also induces VHP.[17–20]

Edema associated with tumor cell infiltration is generally considered to be due to the obstruction of vascular or lymphatic vessels by the tumor cells.[21–24] However, edematous lesions associated with KS cannot solely be ascribed to mechanical obstruction by tumor cells. Microscopic findings of KS lesions include irregular, thin-walled, and dilated vascular spaces associated with extravasated red blood cells, proliferating endothelial cells, perivascular pseudo-granulomatous aggregations of spindle cells, and infiltration of inflammatory cells.[25] Furthermore, some of these abnormal vascular proliferative responses and lymphocytic cell infiltrates are also detected in the clinically uninvolved skin surrounding early flat (macular) AIDS-KS lesions.[26,27] These findings strongly suggest the involvement of soluble factor(s) affecting vascular permeability in the development of edema associated with KS.

To investigate the edematous lesions associated with KS, we have developed an *in vivo* experimental system using nude mouse and long-term culture of spindle cells derived from AIDS-KS lesions (AIDS-KS-cells).[28,29] We have also developed a modified guinea pig system for studying the VHP-inducing activity[30] of the conditioned medium from AIDS-KS cells; this system is highly reproducible and more sensitive and economical than the nude mouse system. Using these model systems, we have investigated the development of edema and the role of cytokines produced by the AIDS-KS cells in promoting VHP.

MATERIALS AND METHODS

Reagents and cytokines Indomethacin, dexamethasone, triprolidine and Evans blue were purchased from Sigma

[3] To whom correspondence should be addressed.

[4] Present address: Division of Immunology, Institute of Gerontology, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki 211

(St. Louis, MO). Recombinant human oncostatin M (rhOSM), recombinant human basic fibroblast growth factor (rhbFGF) and recombinant human vascular endothelial growth factor (rhVEGF) were purchased from R&D (Minneapolis, MN). Recombinant human interleukin-8 (rhIL-8) from Pepro Tech Inc. (Rocky Hill, NJ).

Cell cultures and the conditioned medium (CM) Both short- and long-term cultured AIDS-KS cells were developed from biopsies from a number of HIV-1 infected patients with KS.[28] These tumor cells were derived from pleural fluid (USC-22), lung (KS-3) and oral submucosa (KS-10). These cells were kindly provided by Dr. S. Nakamura, University of Southern California. AIDS-KS cells were subcultured and maintained in gelatinized flasks in RPMI1640 medium supplemented with 15% fetal calf serum and with either 20% CM from an HTLV-II transformed T-cell line (38–10) or 1 ng/ml of rhOSM.[31] The medium was renewed at 3-day intervals. AIDS-KS cells fixed with 0.00125% glutaraldehyde were used as controls in animal experiments.

Non-KS control cells were cultured as follows:human umbilical vein endothelial cells (HUVEC) in gelatinized flasks in RPMI1640 supplemented with 15%FCS, 50 μg/ml endothelial cell growth supplement (ECGS, UBI Inc., Lake Placid, NY) and 45 μg/ml heparin; human smooth muscle cells (HSMC) in RPMI 1640 with 10%FCS; human skin and lung fibroblasts (HSF and HLF) in Dulbecco's modified Eagle's medium with 10%FCS. Media were replaced every 3 days for these cells. These cells were also cultured under the same conditions as AIDS-KS spindle cells.

AIDS-KS-CM was harvested from the confluent KS-3 or USC22 cell cultures (approximately 1.5×10^5 cells/ml). They were then incubated for 72 h with phenol red-free RPMI1640 medium supplemented with either 10% FCS (AIDS-KS-CM10) or 1% bovine serum albumin (BSA) plus 1% NutridomaTM (Boehringer Mannheim, Indianapolis, IN) (AIDS-KS-CM0). The medium was harvested, cells and cell debris were removed by centrifugation, and the supernatant was concentrated 10–30 fold by ultrafiltration (Omega 10K, Filton, MA), then dialyzed against phosphate-buffered saline (PBS), and filtered before storage at 4°C or −20°C. CM from control cell cultures was prepared by the same procedures.

Vascular hyperpermeability (VHP) assays VHP activity induced by AIDS-KS cells or AIDS-KS-CM was evaluated in terms of dye exudation in the local microvasculature. AIDS-KS cells were inoculated intraperitoneally (2.0×10^6) or subcutaneously (4.0×10^6) into 8- to 9-week-old female Balb/c athymic nude (*nu/nu*) mice (Simon Laboratories Inc., Gilroy, CA). Mice were injected intravenously with 100 μl of 1% (w/v) Evans blue in PBS at 30 min, 1 h, 3 h, 6 h, 12 h, 18 h, 24 h, 48 h and 96 h after injection of AIDS-KS cells, then anesthetized with ether and killed 15 min after the dye-injection. The extent of dye (Evans blue) leakage in the mice that had been peritoneally injected with AIDS-KS cells was measured as follows. The peritoneal cavity was lavaged with 1.5 ml of PBS. The amount of Evans blue exuded into the peritoneal fluid was measured in terms of the optical absorbance at 510 nm using a spectrophotometer (Pharmacia LKB Ultrospec II).[32] The dye leakage induced by subcutaneous injection of AIDS-KS cells was visually inspected and photographs were taken for the record.

The VHP activity of AIDS-KS-CM was evaluated as follows. The concentrated AIDS-KS-CM was injected intradermally into 9- to 10-week-old male Hartley guinea pigs (Japan SLC, Hamamatsu) with some modification of the previous method.[33] Guinea pigs were injected intracardially with 1 ml of Evans blue (1% (w/v) in PBS) at various intervals (0–3 h) after the injection of AIDS-KS-CM. Animals were anesthetized with ether and killed 30 min after the dye injection. The dye leakage at the site of AIDS-KS-CM injection was photographed and the dermal lesion was then punched out (2.5 cm in diameter) for dye extraction. The Evans blue was extracted from the skin tissues with formamide at 65°C for 36 h[34] and the absorbance at 510 nm was measured spectrophotometrically.

In order to distinguish the VHP response induced by AIDS-KS cells or the fraction derived from AIDS-KS-CM from that mediated by histamine, triprolidine, a histamine antagonist, was injected (10 μg/site) at the same time as AIDS-KS cells or AIDS-KS-CM.[32] In addition, triprolidine was also injected subcutaneously 8, 10 and 12 h after injection of AIDS-KS cells. To exclude the possibility of involvement of prostaglandins or leukotrienes in mediating the VHP response observed with AIDS-KS cell-derived factor(s), indomethacin or dexamethasone were administered locally (50 μg/kg) or systemically (10 mg/kg intraperitoneally).[35–37] One hundred ng/site of rhbFGF or rhIL-8 was also injected intradermally to examine its ability to induce VHP.

Purification of VHP activity through a heparin affinity column AIDS-KS-CM preparations, thirty ml of 10-fold-concentrated AIDS-KS-CM10 and 10 ml of 30-fold-concentrated AIDS-KS-CM0 (serum-free) were dialyzed overnight against a buffer containing 0.02 *M* Tris. HCl (pH 7.2) and 0.1 *M* NaCl. These CM samples were mixed with 8 ml of heparin-Sepharose (Bio-Rad, Hercules, CA) equilibrated with the dialysis buffer and incubated overnight at 4°C. The mixture was packed into a disposable econo column (Bio-Rad). The flow-through fraction was collected and further concentrated 3-fold. The column was extensively washed with the starting buffer (0.02 *M* Tris. HCl (pH 7.2) and 0.1 *M* NaCl) and was eluted from the column in a step-wise manner (0.5

M, 0.8 *M*, 2.0 *M* and 3.0 *M* NaCl). Ten ml fractions of bound material from each step were collected and dialyzed against PBS.

Neutralization of VHP activity by monospecific anti-human $VEGF_{165}$ rabbit serum One ml of the 0.5 *M* NaCl fraction from the heparin-affinity column was incubated overnight at 4°C with anti-human $VEGF_{165}$ rabbit serum (a generous gift from Dr. H.A. Weich, Geselshaft für Biotechnologische Forschung, Germany) at different dilutions (1:10, 1:20 and 1:50 dilution).[38] This antiserum neutralized the VHP activity induced by the rhVEGF. Fifty μl of this antiserum completely neutralized the VHP activity of 50 ng of VEGF in the guinea pig model described above (our unpublished observations). Each fraction from the heparin-affinity column was first incubated with rabbit anti-human VEGF antiserum overnight at 4°C and the VEGF-anti-VEGF complex was excluded by incubation with protein A Sepharose beads (Bio-Rad) followed by centrifugation. The residual VHP activity after immunoprecipitation of VEGF was examined by dye leakage assay using the guinea pig model as described.

Immunohistochemical staining of VEGF AIDS-KS cells and thin sections of KS tissue (stomach) of a 59-year-old Japanese male AIDS-KS case were stained with anti-human VEGF rabbit serum (Santa Cruz Biotechnology

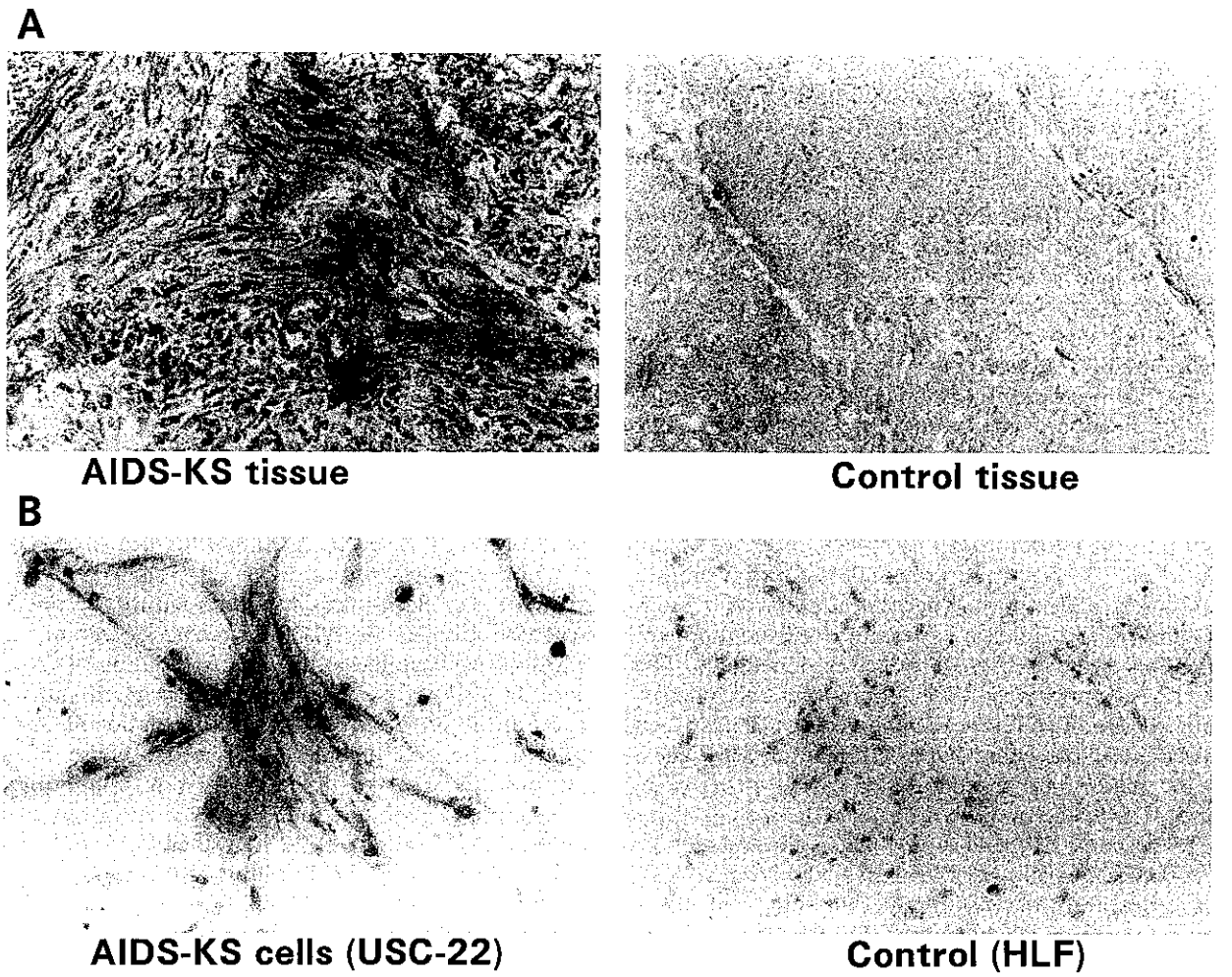

Fig. 1. Immunostaining of fresh KS tissue and AIDS-KS cells with rabbit polyclonal antibody to VEGF. A, Tissue sections from fresh frozen AIDS-KS stomach lesion from a 59-year-old male case and control stomach tissue from a healthy individual were incubated with 1:100 diluted anti-VEGF rabbit serum at 37°C for 45 min, then biotinylated anti-rabbit immuno-globulin was added followed by incubation with peroxidase-conjugated streptavidin at 37°C for 45 min. Colorization was performed by using a commercial kit (sABC system, Dako). Note that the spindle-shaped KS cells were stained with anti-VEGF, while no positive cells were detected in control tissue. B, AIDS-KS (USC-22) and control human lung fibroblast (HLF) cells were incubated with the same primary antibody and stained in the same way as in A.

Inc., Santa Cruz, CA). Following pretreatment in methanol with 0.3% hydrogen peroxide for 20 min to inactivate endogenous peroxidase activity, the sections were rinsed with PBS and preincubated with PBS with 1% bovine serum albumin and 1% swine serum albumin. Sections were incubated with anti VEGF rabbit serum as the primary antibody or with normal rabbit serum (as a negative control) at 37°C for 45 min in a moist chamber, then reacted with biotinylated anti-rabbit immunoglobulin followed by peroxidase-conjugated streptavidin at 37°C for 45 min. Colorization was performed by using a commercial kit (sABC system, Dako, Glostrup, Denmark).

RESULTS

Immunostaining of VEGF in AIDS-KS tissue and cultured AIDS-KS cells Production of VEGF was examined in AIDS-KS cells and AIDS-KS tissue using immunohistochemical staining with anti-VEGF antibody. Fig. 1 demonstrates that VEGF is synthesized in fresh AIDS-KS tissue as well as in AIDS-KS (USC-22) cells, while control tissue and control (HLF) cells were negative for VEGF production. Other AIDS-KS cells, KS-3 and KS-10, were similarly stained with anti-VEGF antibody, while other control cells, HUVEC and HSF, were negative (data not shown).

Induction of VHP in nude mice by inoculation of AIDS-KS cells To examine if AIDS-KS cells produce a substance that induces edema, cultured AIDS-KS3 cells were inoculated intraperitoneally into nude mice. After various times of inoculation of AIDS-KS3 cells, VHP was assessed by measuring the amount of Evans blue exuded into the peritoneal cavity. Evans blue dye (1 mg in 100 μl of PBS per capita) was injected intravenously 15 min before collecting the peritoneal fluid. As demonstrated in Fig. 2, a biphasic pattern of dye exudation was observed only when AIDS-KS cells were inoculated: The first VHP peak appeared at 15–30 min after cell inoculation and the second VHP peak appeared at 12 h after cell inoculation. On the other hand, HUVEC or HSF cells, when inoculated into the peritoneal cavity in the same way as AIDS-KS cells, did not produce the second phase of VHP response. Furthermore, when glutaraldehyde-fixed AIDS-KS cells were inoculated, the second VHP response was not observed. It was thus suggested that a substance(s) produced by live AIDS-KS cells was responsible for the edema associated with the KS lesion.

Similarly, AIDS-KS3 cells were subcutaneously inoculated into nude mice. The local dye exudation was observed after intravenous injection of Evans blue by dissecting the dermal site of AIDS-KS cell inoculation. As shown in Fig. 3, A and B, dye exudation was demonstrated in the skin tissue surrounding the site of AIDS-KS cell inoculation after 30 min (1st phase VHP response) and 12 h (2nd phase VHP response). Spindle cells obtained from fresh biopsy tissues of AIDS-KS patients also elicited a biphasic response (data not shown). To clarify this biphasic nature of the VHP response induced by AIDS-KS cells, we examined the effect of a histamine antagonist, triprolidine (Fig. 3, C and D). Ten μg of triprolidine was injected subcutaneously at the site of AIDS-KS cell inoculation at the same time as the cell inoculation. The 1st phase of VHP response, as observed in terms of Evans blue exudation, was blocked by triprolidine (Fig. 3C). However, the 2nd phase of VHP response (12 h) was not blocked even by repeated injections of triprolidine (three times at 8 h, 10 h, and 12 h) after inoculation (Fig. 3D). The 1st VHP response evoked by inoculations of HUVEC, HSF, and glutaraldehyde-fixed AIDS-KS cells could also be blocked by triprolidine (data not shown).

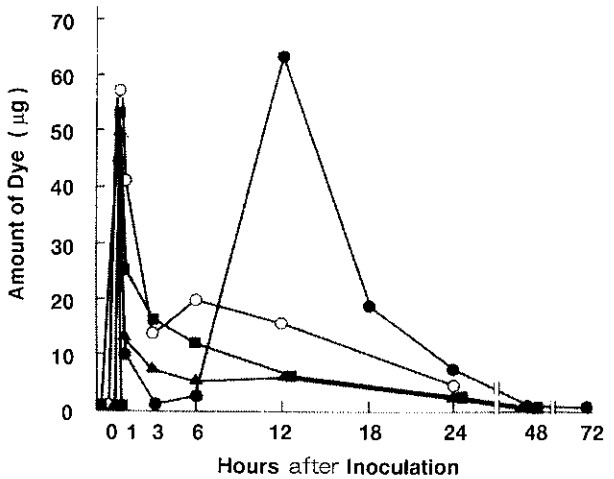

Fig. 2. Kinetics of VHP response induced by intraperitoneal inoculation of AIDS-KS and control cells in nude mice. Various cells (2×10^6) were intraperitoneally injected into nude mice. The VHP response was measured according to the procedures described in "Materials and Methods" at 0 h, 0.25 h, 0.5 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h after cell inoculation. Experiments were repeated twice with at least 4 mice in each case. Note that the biphasic VHP response, early (30 min) and late (12 h), was observed only in the case of AIDS-KS cells. The standard deviation of the data (SD) was within the range of ±15%. (●), AIDS-KS3 cells; (■), HUVEC; (▲), HSF; (○), 0.00125% glutaraldehyde-fixed AIDS-KS3 cells.

Induction of VHP response in guinea pigs by inoculation of conditioned medium from AIDS-KS cells We then examined whether the cell culture supernatant of AIDS-KS cells had a similar effect. The conditioned medium from AIDS-KS3 cell culture, AIDS-KS-CM0 or AIDS-

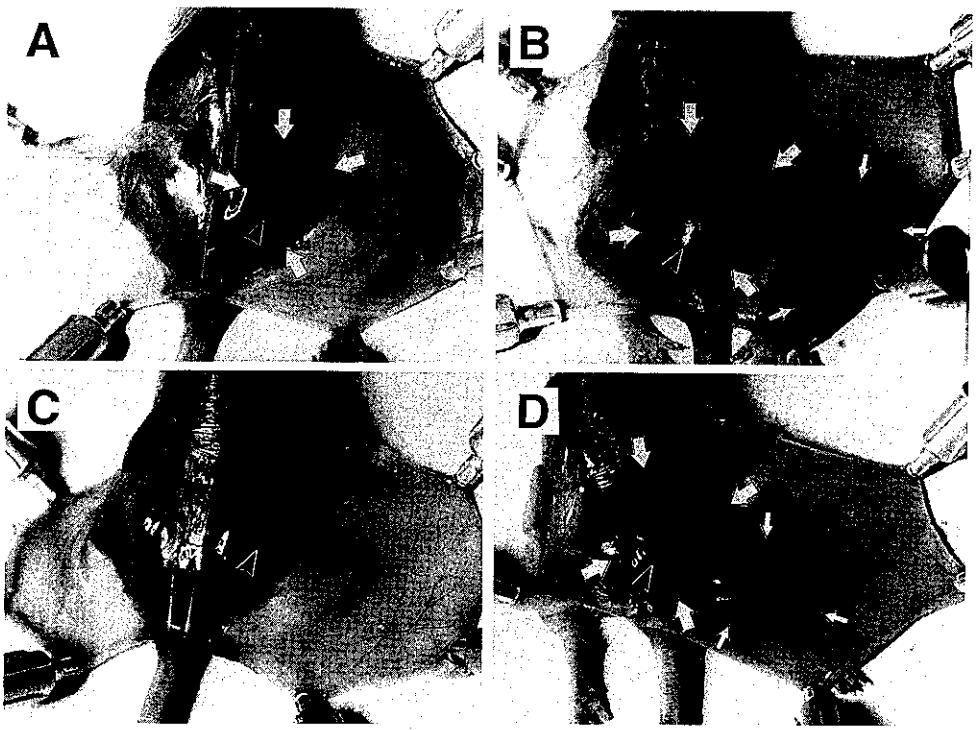

Fig. 3. Effect of histamine antagonist on the early and late vascular permeability responses induced by AIDS-KS cells. AIDS-KS3 cells (4×10^6) were inoculated subcutaneously into the backs of nude mice. Early (30 min) and late (12 h) vascular permeability responses are shown in panels A and B, respectively. Experiments were performed twice, each with 4 mice, for each data point. The effects of local administration of 10 μg of triprolidine were examined (in panels C and D). The early-phase response was completely inhibited by triprolidine treatment while the late-phase was resistant to repeated triprolidine treatment at the initial time and again at 8 and 12 h after transplantation. Closed arrowheads indicate the site of transplantation of the AIDS-KS cells. The open arrows indicate the area of dye exudation. A, dye exudation at 30 min after inoculation; B, dye exudation at 12 h after inoculation; C, dye exudation at 30 min after inoculation (with single triprolidine treatment); D, dye exudation at 12 h after inoculation (with triprolidine treatment 3 times).

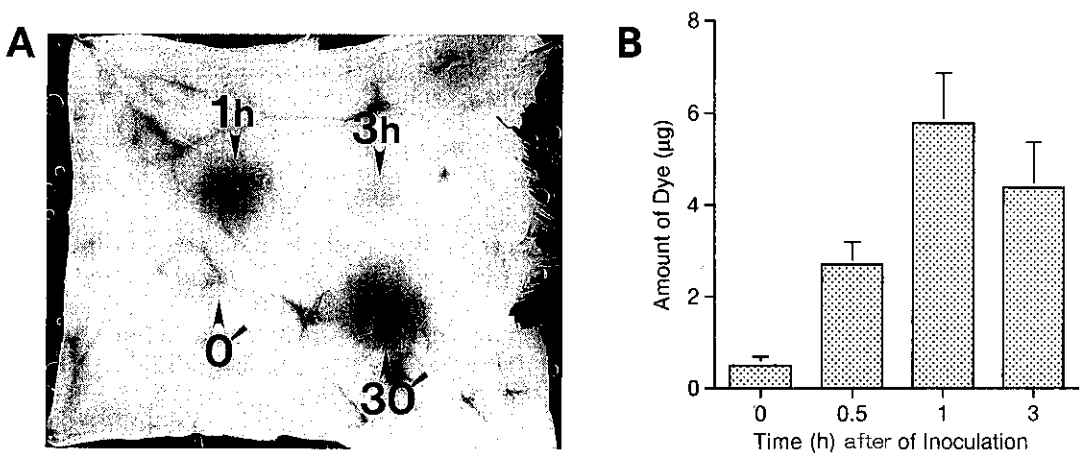

Fig. 4. Induction of VHP by concentrated AIDS-KS-CM in guinea pig skin. A, Two hundred μl of the AIDS-KS10-CM was injected intradermally into the back of guinea pigs at 0 min, 30 min, 1 h and 3 h after local administration of triprolidine (10 μg/site) ($n=4$). A representative picture is shown in A. Evans blue was intracardially administered 30 min after the inoculation of samples. B, Animals were killed under anesthesia 30 min after the dye injection, and leaked dye in the skin was extracted with formamide and measured spectrophotometrically as described.[33] Standard deviation bars are indicated.

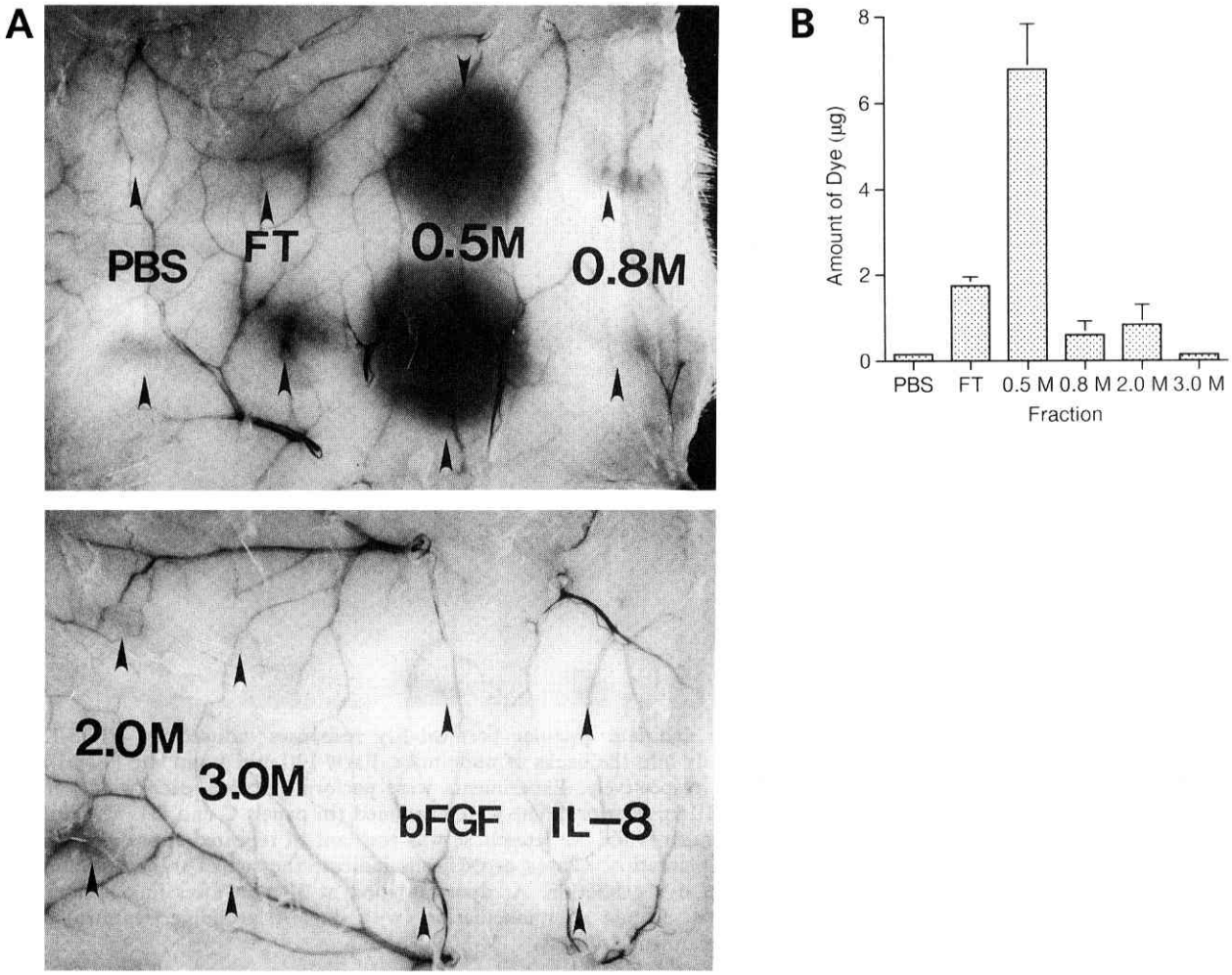

Fig. 5. Fractionation of the VHP activity in AIDS-KS-CM on a heparin affinity column. The affinity-purified and 10-fold-concentrated AIDS-KS10-CM was injected intradermally into the back of guinea pigs (200 μl each of flow-through (FT), 0.5 *M*, 0.8 *M*, 2.0 *M* or 3.0 *M* NaCl fraction, $n=4$). The amount of the injected sample corresponded to the culture supernatant of 2×10^5 AIDS-KS cells. Triprolidine was also administered locally (10 μg/site). Evans blue was intracardially administered at 30 min after sample injection. A, Dye exudation observed with each fraction. A representative result is demonstrated. B, Quantitation of the amount of exuded dye extracted with formamide from each skin region of guinea pigs. The amount of Evans blue dye was measured as described.[33] Error bars show SD.

KS-CM10 was concentrated 10–30 fold by passing it through a filter (thus removing small molecular weight substances of less than 10000 daltons) and inoculated intradermally into guinea pigs. To preclude the involvement of histamine, triprolidine was injected concomitantly. As shown in Fig. 4, the VHP response was observed after injection of the AIDS-KS cell supernatant. The concentrated AIDS-KS-CM10 induced maximum VHP at 1 h after injection. The effect tapered off after 3 h. The control conditioned medium from HUVEC and HSF cells, prepared similarly to that of AIDS-KS cells, did not show any VHP response (data not shown). Since this VHP response was observed in the presence of the histamine antagonist, it was considered to be equivalent to the triprolidine-resistant VHP response observed when AIDS-KS cells were inoculated (Figs. 2 and 3), although the AIDS-KS cell supernatant elicited VHP as early as 1 to 3 h after injection while AIDS-KS cell inoculation into nude mice caused VHP at 12 h after injection.

Fractionation of the VHP-inducing activity in AIDS-KS-CM on a heparin affinity column The concentrated AIDS-KS-CM10 was fractionated by heparin-affinity column chromatography and the VHP-inducing activity of each fraction was examined with the guinea pig system

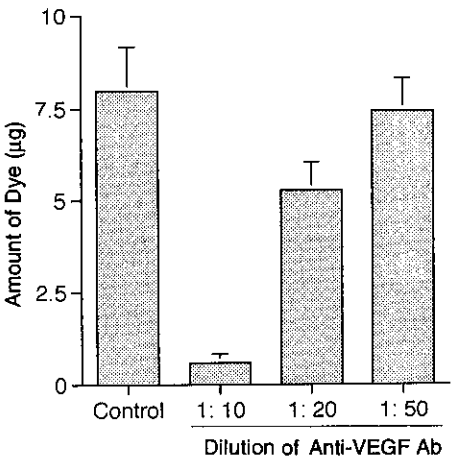

Fig. 6. Neutralization of the VHP-inducing activity in partially purified AIDS-KS-CM by anti-VEGF165 antibody. The 0.5 *M* NaCl fraction (200 μl) from the heparin affinity column with the VHP activity (Fig. 5) was preincubated with preimmune serum or anti-VEGF serum, immunoprecipitated with protein A and intradermally injected into guinea pigs. The VHP activity was measured at 30 min after injection. Exuded dye in the skin was extracted and measured as in Figs. 4 and 5 ($n=4$). Error bars show SD. Control, treated with preimmune rabbit serum and protein A. Amounts of rabbit anti-VEGF neutralizing antibody are indicated in the figure.

(Fig. 5). The exuded dye was extracted from the excised skin lesion and was measured by spectrophotometry. As diagramatically illustrated in Fig. 5A, the major VHP response was induced by the injection of the 0.5 *M* NaCl-eluate fraction. The flow-through fraction showed some residual activity, which might be due to the actions of IL-1 beta and IL-6, non-heparin binding factors. Control (PBS with BSA) and other heparin-binding fractions (0.8 *M*, 2.0 *M* and 3.0 *M* NaCl eluate) did not induce VHP response (Fig. 5A). Since basic fibroblast growth factor (bFGF) and IL-8 are known to be produced by AIDS-KS cells,[14, 39] we injected recombinant human bFGF and IL-8 intradermally into guinea pigs (500 ng/site). However, bFGF and IL-8 did not induce the histamine-independent VHP response. Fig. 5B showed that the heparin-purified fraction (0.5 *M* NaCl fraction of AIDS-KS-CM10) induced the VHP response. The temporal profile of the VHP response elicited by the 0.5 *M* NaCl fraction of AIDS-KS-CM10 was examined. The VHP response was induced within 10 min, reached its peak at 30 min and then tapered off sharply (data not shown), which was comparable with the result using the crude sample (Fig. 4).

We then examined the effect of anti-VEGF antibody on the VHP response elicited by the AIDS-KS-CM10 fraction. The 0.5 *M* NaCl fraction from AIDS-KS-CM10 was incubated with anti-human VEGF165 rabbit serum, then the VEGF-anti-VEGF immune complex was removed by immunoprecipitation and injected into guinea pig skin. As shown in Fig. 6, anti-VEGF antibody neutralized the VHP-inducing activity of AIDS-KS-CM10 in a dose-dependent manner. In contrast, the pre-immune rabbit serum did not inhibit the VHP-inducing activity.

DISCUSSION

The data presented here indicate that VEGF is produced by AIDS-KS cells and is responsible for VHP induction in KS lesions. Although multiple soluble factors are known to be produced by AIDS-KS cells,[14, 39, 40] this study clearly showed that VEGF has a primary role in the VHP response associated with KS.

In an attempt to investigate the nature and possible cause of the edematous lesions associated with KS, we have developed *in vivo* assay systems using nude mice and guinea pigs. Our present study has revealed that AIDS-KS cells, when transplanted intraperitoneally or subcutaneously, induced massive exudation of injected Evans blue dye in nude mice. A typical biphasic pattern of the VHP response was observed: the early histamine-dependent response and the late histamine-independent response. This character of AIDS-KS cells is intrinsic to KS, since fresh KS tissues from patients also induce edema when transplanted into nude mice (our unpublished observations). Similarly, when CM from AIDS-KS cells was injected intradermally into the skin of guinea pigs, the VHP response was observed in the presence of triprolidine, a histamine antagonist. Upon fractionation through a heparin-affinity column, the VHP-inducing activity was recovered in the 0.5 *M* NaCl fraction. This activity was abolished by preincubation with anti-VEGF antibody, suggesting that VEGF is primarily responsible for the VHP response associated with KS.

The histamine-independent VHP response induced by AIDS-KS cell transplantation showed the peak effect at 12 h after transplantation in nude mice, while crude or heparin-purified CM from AIDS-KS cells showed the maximum VHP response 30 to 60 min post-inoculation in nude mice and guinea pigs. This difference of the temporal profile may reflect the time lag during which KS cells accumulated and secreted sufficient VEGF to induce VHP, whereas CM enriched VEGF might have exerted the VHP response without delay.

Induction of VHP response by VEGF (also known as vascular permeability factor (VPF)) was initially demonstrated with human glioblastoma by Ohnishi *et al.*[37] They showed that the VHP response was inhibited either by lipoxygenase inhibitors or by glucocorticoid, but not by

anti-histamines or cyclooxygenase inhibitors such as indomethacin. We also found that the VHP response induced by KS cells was inhibited by high, non-physiological doses of dexamethasone (10 mg/kg), but not by high doses of indomethacin (data not shown). Therefore, a common mechanism is involved in the edema associated with KS and glioblastoma.

Among the cytokines known to be produced by AIDS-KS cells, VEGF is of special interest in terms of KS pathogenesis. VEGF is a member of the platelet-derived growth factor (PDGF) family[41, 42] and is also produced by a number of human and rodent tumor cell lines.[43] Since VEGF is reported to promote the growth of vascular endothelial cells, as well as to induce VHP in animal models,[17–20, 41, 44] it may have a general role in neovascularization and edema induction, and thus may play a central role in tumor progression and possibly also tumor invasion and metastasis of malignant neoplasms.

Based on our present data we suggest that AIDS-KS cells may mediate significant VHP via VEGF production. Edema in AIDS-KS patients may be a result of constitutive VEGF production associated with KS cell proliferation and its tissue infiltration. The efflux of fluids, proteins, other nutrients and proinflammatory cytokines such as tumor necrosis factor-α from post-capillary venules that are induced by VEGF is also considered to be involved in the extravascular matrix formation and angiogenesis in KS lesions.[45] The observations reported here support the hypothesis that the clinical picture of AIDS-KS lesions is generated by the cytokines produced by AIDS-KS cells.

ACKNOWLEDGMENTS

We thank S. Nakamura for providing AIDS-KS cells and K. Asamitsu and H. Mohri for technical support. This work was supported by Grants-in-Aid from the Ministry of Health and Welfare and the Ministry of Education, Science and Culture, Japan.

(Received June 28, 1996/Accepted August 26, 1996)

REFERENCES

1) Folkman, J. and Shing, Y. Angiogenesis. *J. Biol. Chem.*, **267**, 10931–10934 (1992).
2) Dovorak, H. F., Orenstein, N. S., Carvalho, A. C., Churchill, W. H., Dovorak, A. M., Galli, S. J., Feder, J., Bitzer, A. M., Rypysc, J. and Giovinco, P. Induction of a fibrin-gel investment: an early event in line 10 hepatocarcinoma growth mediated by tumor-secreted products. *J. Immunol.*, **122**, 166–174 (1979).
3) Dovorak, H. F., Senger, D. R., Dovorak, A. M., Harvey, V. S. and McDonagh, J. Regulation of extravascular coagulation by microvascular permeability. *Science*, **227**, 1059–1061 (1985).
4) Scully, P. A., Steinman, H. K., Kennedy, C., Trueblood, K., Frisman, D. M. and Voland, J. R. AIDS-related Kaposi's sarcoma displays differential expression of endothelial surface antigens. *Am. J. Pathol.*, **130**, 244–251 (1988).
5) Roth, W. K., Werner, S., Risau, W., Remberger, K. and Hofschneider, P. H. Cultured, AIDS-related Kaposi's sarcoma cells express endothelial cell markers and are weakly malignant *in vitro*. *Int. J. Cancer*, **42**, 767–773 (1988).
6) Yang, G. C. H., Brooks, J. J., Roberts, S. and Gupta, P. K. The detection of acquired immunodeficiency syndrome-associated Kaposi sarcoma cells in pleural effusion by CD34 immunostain. *Cancer*, **72**, 2260–2265 (1993).
7) Wittek, A. E., Mitchell, C. D., Armstrong, G. R., Albini, A., Martin, G. R., Seemann, R., Levenbook, I. S., Wierenga, D. E., Ridge, J., Dunlap, R. C., Lundquist, M. L., Steis, R. G., Longo, D. L., Muller, J. and Quinnan, Jr., G. V. Propagation and properties of Kaposi's sarcoma-derived cell lines obtained from patients with AIDS: similarity of cultured cells to smooth muscle cells. *AIDS*, **5**, 1485–1493 (1991).
8) Weich, H. A., Salahuddin, S. Z., Gill, P., Nakamura, S., Gallo, R. C. and Folkmann, J. AIDS-associated Kaposi's sarcoma-derived cells in long-term culture express and synthesize smooth muscle alpha-actin. *Am. J. Pathol.*, **139**, 1251–1258 (1991).
9) Kaposi, M. Idiopathisches multiples pigmentsarkom der Haut. *Arch. Dermatol. Syphilol.*, **4**, 265–273(1872).
10) Ognibene, F. P., Steis, R. G., Macher, A. M., Liotta, L., Gelmann, E., Pass, H. I., Lane, H. C., Fauci, A. S., Parrillo, J. E., Masur, H. and Shelhamer, J. H. Kaposi's sarcoma causing pulmonary infiltrates and respiratory failure in the acquired immunodeficiency syndrome. *Ann. Intern. Med.*, **102**, 471–475 (1985).
11) Biggs, B. A., Crowe, S. M., Lucas, C. R., Ralston, M., Thompson, I. L. and Hardy, K. J. AIDS related Kaposi's sarcoma presenting as ulcerative colitis and complicated by toxic megacolon. *Gut*, **28**, 1302–1306 (1987).
12) Novis, B. H., King, H. and Bank S. Kaposi's sarcoma presenting with diarrhea and protein-losing enteropathy. *Gastroenterology*, **67**, 996–1000 (1974).
13) Krown, S. E. Editorial review: AIDS-associated Kaposi's sarcoma: pathogenesis, clinical course and treatment. *AIDS*, **2**, 71–80 (1988).
14) Ensoli, B., Nakamura, S., Salahuddin, S. Z., Biberfeld, P., Larsson, L., Beaver, B., Wong-Staal, F. and Gallo, R. C. AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. *Science*, **243**, 223–226 (1989).
15) Xerri, L., Hassoun, J., Planche, J., Guigou, V., Grob, J.-J., Parc, P., Birnbaum, D. and De Lapeyriere, O. Fibroblast

growth factor gene expression in AIDS-Kaposi's sarcoma detected by *in situ* hybridization. *Am. J. Pathol.*, **138**, 9–15 (1991).
16) Weindel, K., Marmé, D. and Weich, H. A. AIDS-associated Kaposi's sarcoma cells in culture express vascular endothelial growth factor. *Biochem. Biophys. Res. Commun.*, **183**, 1167–1174 (1992).
17) Connolly, D. T., Heuvelman, D. M., Nelson, R., Olander, J. V., Eppley, B. L., Delfino, J. J., Siegel, N. R., Leimgruber, R. M. and Feder, J. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. *J. Clin. Invest.*, **84**, 1470–1478 (1989).
18) Leung, D. W., Cachianes, G., Kuang, W.-J., Goeddel, D. V. and Ferrara, N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*, **246**, 1306–1309 (1989).
19) Tischer, E., Mitchell, R., Hartman, T., Silva, M., Gospondarowicz, D., Fiddes, J. C. and Abraham, J. A. The human gene for vascular endothelial growth factor. *J. Biol. Chem.*, **266**, 11947–11954 (1991).
20) Houck, K. A., Leung, D. W., Rowland, A. M., Winer, J. and Ferrara, N. Dual regulation of vascular endothelial growth factor bioavailability by genetic and proteolytic mechanisms. *J. Biol. Chem.*, **267**, 26031–26037 (1992).
21) Rothmann, S. Some clinical aspects of Kaposi's sarcoma in the European and North American population. *Acta Unio Int. Cancrum*, **18**, 364–371 (1962).
22) Mitsuyasu, R. T. and Groopman, J. E. Biology and therapy of Kaposi's sarcoma. *Sem. Oncol.*, **11**, 53–59 (1984).
23) Kyalwazi, S. K. Kaposi's sarcoma: clinical features, experience in Uganda. *Antibiot. Chemother.*, **29**, 59–67 (1981).
24) Conant, M. A. Cutaneous neoplasia associated with the acquired immunodeficiency syndrome (AIDS). *In* "IV Cancer of the Skin," ed. R. L. Friedman, pp. 307–316 (1991). Saunders, Philadelphia.
25) Ackerman, A. B., Gottlieb, G. J. Atlas of the gross and microscopic features. *In* "Kaposi's Sarcoma: a Text and Atlas," ed. G. J. Gottlieb and A. B. Ackerman, pp. 29–71 (1988). Lea & Febiger, Philadelphia.
26) De Dobbeleer, G., Godfrine, S., André, J., Ledoux, M. and De Maubeuge, J. Clinically uninvolved skin in AIDS: evidence of atypical dermal vessels similar to early lesions observed in Kaposi's sarcoma. *J. Cutaneous Pathol.*, **14**, 154–157 (1989).
27) Ruszczak, Z., da Silva, A. M. and Orfanos, C. E. Angioproliferative changes in clinically noninvolved, perilesional skin in AIDS-associated Kaposi's sarcoma. *Dermatologia*, **175**, 270–279 (1987).
28) Nakamura, S., Salahuddin, S. Z., Biberfeld, P., Ensoli, B., Markham, P. D., Wong-Staal, F. and Gallo, R. C. Kaposi's sarcoma cell: long-term culture with growth factor(s) from retrovirus infected CD4$^+$ T-cells. *Science*, **242**, 426–430 (1988).
29) Salahuddin, S. Z., Nakamura, S., Biberfeld, P., Kaplan, M. H., Markham, P. D., Larsson, L. and Gallo, R. C. Angiogenic properties of Kaposi's sarcoma-derived cells after long-term culture *in vitro*. *Science*, **242**, 430–433 (1988).
30) Nakamura, S., Sakurada, S., Salahuddin, S. Z., Osada, Y., Tanaka, N. G., Sakamoto, N., Sekiguchi, M. and Gallo, R. C. Inhibition of development of Kaposi's sarcoma-related lesions by a bacterial cell wall complex. *Science*, **255**, 1437–1440 (1992).
31) Radoka, S. F., Nakamura, S., Sakurada, S. and Salahuddin, S. Z. Correlation of oncostatin M secretion by human retrovirus infected cells with potent growth stimulation of cultured spindle cells from AIDS-Kaposi's sarcoma. *J. Immunol.*, **150**, 5195–5201 (1993).
32) Hayashi, H., Yoshinaga, M., Koono, M., Miyoshi, H. and Matsumura, M. Endogenous permeability factors and their inhibitors affecting vascular permeability in cutaneous arthus reaction and thermal injury. *Br. J. Exp. Pathol.*, **45**, 419–435 (1964).
33) Miles, A. A. and Miles, E. M. Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea-pigs. *J. Physiol.*, **118**, 228–257 (1952).
34) Udaka, K., Takeuchi, Y. and Movat, H. Z. Simple method for quantitation of enhanced vascular permeability. *Proc. Soc. Exp. Biol. Med.*, **133**, 1384–1387 (1970).
35) Senger, D. R., Galli, S. J., Dovorak, A. M., Perruzzi, C. A., Harvey, V. S. and Dovorak, H. F. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*, **219**, 983–985 (1983).
36) Criscuolo, G. R., Merrill, M. J. and Oldfield, E. H. Further characterization of malignant glioma-derived vascular permeability factor. *J. Neurosurg.*, **69**, 254–262 (1988).
37) Ohnishi, T., Sher, P. B., Posner, J. B. and Shapiro, W. R. Capillary permeability factor secreted by malignant brain tumor. Role in peritumoral brain edema and possible mechanism for anti-edema effect of glucocorticoids. *J. Neurosurg.*, **72**, 245–251 (1990).
38) Fiebich, B. L., Jäger, B., Schöllmann, C., Weindel, K., Wilting, J., Kochs, G., Marmé, D., Hug, H. and Weich, H. A. Synthesis and assembly of functionally active human vascular endothelial growth factor homodimers in insect cells. *Eur. J. Biochem.*, **211**, 19–26 (1993).
39) Sciacca, F. L., Stürzl, M., Bussolino, F., Sironi, M., Brandstetter, H., Zietz, C., Zhou, D., Matteucci, C., Peri, G., Sozzani, S., Benelli, R., Arese, M., Albini, A., Colotta, F. and Mantovani, A. Expression of adhesion molecules, platelet-activating factor, and chemokines by Kaposi's sarcoma cells. *J. Immunol.*, **153**, 4816–4825 (1994).
40) Ensoli, B., Barillari, G. and Gallo, R. C. Cytokines and growth factors in the pathogenesis of AIDS-associated Kaposi's sarcoma. *Immunol. Rev.*, **127**, 147–155 (1992).
41) Keck, P. J., Hauser, S. D., Krivi, G., Sanzo, K., Warren, T., Feder, J. and Connoly, D. T. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science*, **246**, 1309–1312 (1989).
42) Conn, G., Bayne, M. L., Soderman, D. D., Kwok, P. W., Sullivan, K. A., Palisi, T. M., Hope, D. A. and Thomas,

K. A. Amino acid and cDNA sequences of a vascular endothelial cell mitogen that is homologous to platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA,* **87,** 2628–2632 (1990).

43) Senger, D. R., Perruzzi, C. R., Feder, J. and Dovorak, H. F. A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. *Cancer Res.,* **46,** 5629–5632 (1986).

44) Conolly, D. T., Olander, J. V., Heuvelman, D., Nelson, R., Monsell, R., Siegel, N., Haymore, B. L., Leimgruber, R. and Feder, J. Human vascular permeability factor, isolation from U937 cells. *J. Biol. Chem.,* **264,** 20017–20024 (1989).

45) Koolwijk, P., van Erk, M. G. M., de Vree, W. J. A., Vermeer, M. A., Weich, H. A., Hanemaaijer, R. and van Hinsbergh, V. W. M. Cooperative effect of TNF-α, bFGF, and VEGF on the formation of tubular structures of human microvascular endothelial cells in a fibrin matrix. Role of urokinase activity. *J. Cell Biol.,* **132,** 1177–1188 (1996).