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Interaction of Interleukin-1 and Interferon-γ on Fibroblast Growth Factor-induced Angiogenesis

Ken-ichi Norioka,[1,3] Toshihiro Mitaka,[1] Yohichi Mochizuki,[1] Masako Hara,[2,4] Mitsuhiro Kawagoe[2] and Haruo Nakamura[2]

[1]*Department of Pathology, Cancer Research Institute, Sapporo Medical University, School of Medicine, South 1, West 17, Chuo-ku, Sapporo 060 and* [2]*First Department of Internal Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa 359*

The interaction of interleukin-1 (IL-1) and interferon-γ (IFN-γ) actions on several aspects of angiogenesis *in vitro* and *in vivo* was studied. The proliferation and migration of human umbilical vein endothelial cells cultured with basic fibroblast growth factor (bFGF) were synergistically inhibited by cotreatment with IL-1 and IFN-γ. Endothelial cell adhesion to collagen was suppressed by IL-1 and the effect was slightly enhanced by the combination of IL-1 and IFN-γ. Local administration of IL-1 (10,000 U) and IFN-γ (1,000 U) inhibited bFGF-induced angiogenesis in the skin of mice, and synergistic inhibitory activity of the combination was demonstrated. Expression of FGF receptors was strongly downregulated by the combination, whereas expressions of epidermal growth factor (EGF) receptors, integrin β_1 and integrin β_3 were not. EGF partially abrogated the growth-inhibitory effects of IL-1 and IFN-γ. These findings indicate that IL-1 and IFN-γ are each able to act an angiogenesis inhibitor in a situation where FGF plays a major role in angiogenesis, and the activity is synergistically enhanced when they are used in combination.

Key words: Interleukin-1 — Interferon-γ — Fibroblast growth factor — Vascular endothelial cell — Angiogenesis

Angiogenesis is highly regulated under normal conditions such as reproduction, embryonic development and repair. However, in many pathological states, persistent and unabated angiogenesis appears as a result of failure of this regulation. Diseases that are supposed to be angiogenesis-dependent include rheumatoid arthritis, diabetic retinopathy, and tumor development.[1] Conversely, these diseases might be controlled by regulation of angiogenesis. Various endogenous molecules may be concerned in the regulation. Angiostatic steroids and protamine were the first such compounds to be identified as angiogenesis inhibitors.[2] Several cytokines are also candidates. Interferon-γ (IFN-γ) has been shown to inhibit the proliferation of vascular endothelial cells (EC)[3] and angiogenesis *in vitro*,[4] and it also partially inhibits the vascular response in mice by suppression of the signal initiated by allogenic lymphocytes.[5] Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF) inhibit the proliferation of EC *in vitro*,[6,7] but they have complex actions and do not necessarily inhibit angiogenesis *in vivo*.[8–11] The reason for the discrepancy between the *in vitro* and *in vivo* observations is unclear. Since more than one angiogenic factor may be involved in angiogenesis, and simultaneous exposure to more than one cytokine occurs *in vivo*,[12] it is necessary to examine the interaction of these factors in angiogenesis to understand the role of cytokines *in vivo*, and ultimately perhaps to utilize them for therapy.

A previous study has established that IL-1, IFN-γ, and TNF, acting synergistically at low, subthreshold concentrations, are able to inhibit completely the proliferation of mouse aortic EC *in vitro*.[13] In the present study, we focused on the interaction of IL-1 and IFN-γ, and by using human EC *in vitro*, we examined their effects on several aspects of angiogenesis, including related receptor expressions on EC by staining with specific antibodies. Their effect on angiogenesis induced by basic fibroblast growth factor (bFGF) *in vivo* was also investigated in the skin of mice.

MATERIALS AND METHODS

Peptide growth factors Reagents included recombinant human IL-1α (2.3×10^7 U/mg, endotoxin content <0.32 ng/mg protein; Dainippon Pharmaceutical Co., Osaka); recombinant human IFN-γ (2.0×10^7 U/mg, endotoxin content <0.75 ng/mg protein; Shionogi Pharmaceutical Co., Osaka); recombinant mouse IFN-γ (4.5–9.0×10^6 U/mg; Genzyme, USA); recombinant bFGF (Takeda Chemical Ind., Osaka); mouse epidermal growth factor (EGF; Collaborative Research, USA).

Cell culture EC were collected from the umbilical cord vein as previously described.[14] The cells were stained

[3] To whom reprint requests and correspondence should be addressed.

[4] Present address: Institute of Rheumatology, Tokyo Women's Medical College, KS Bldg., 9-12 Wakamatsu-cho, Shinjuku-ku, Tokyo 162.

with anti-factor VIII-related antigen (DAKO, USA) but not with anti-desmin antibody (DAKO). Ultrastructurally, Weibel-Palade bodies were identified in the cytoplasm. The cells were cultured in Medium 199 (GIBCO, USA) containing 10% heat-inactivated fetal calf serum (FCS; Hyclone, USA), 0.1 m*M* L-ascorbic acid phosphate magnesium salt (Wako, Osaka), 2 ng/ml of bFGF and antibiotics on dishes coated with rat-tail collagen.

Proliferation assay EC (passages 3–10) were plated at a concentration of 1×10^4 per well in 24-well plates precoated with gelatin. The next day, test reagents were added to the cultures. At subsequent time points, cells were harvested by trypsinization and counted with an electronic particle counter (Erma, Tokyo).

Cell migration assay The migration assay was performed according to the method of Defilippi *et al.*[15] with some modification. Confluent monolayers of EC in 24-well plates precoated with gelatin were treated for 48 h with IL-1 and/or IFN-γ in medium containing 10% FCS and 2 ng/ml of bFGF. Cells were then treated for 20 min with 40 μg/ml of mitomycin C (Kyowa, Tokyo) to block DNA synthesis; at this concentration cell division was blocked by 95% as determined by counting the cell number after a 3-day incubation. After being washed with phosphate-buffered saline (PBS), the monolayers were wounded with a plastic pipette tip. The cells were washed to eliminate cellular debris, allowed to migrate into the free space for 16 h in the presence of the same mediators, fixed with 3% paraformaldehyde, stained with Giemsa and photographed. Migration was quantitated by counting the number of cells within 1 mm from the wound edge using a light microscope with an ocular grid.

Cell adhesion assay EC attachment to collagen-coated substrates was measured by using the method of Asakura *et al.*[16] with some modifications. Twenty-four-well plates were coated with 50 μg of rat tail collagen or purified bovine collagen (Koken, Tokyo). The cells for the assays were treated for 48 h with IL-1 and/or IFN-γ in medium containing 2 ng/ml of bFGF and were then detached from the culture flasks by trypsinization. The cells were pelleted and then resuspended in M199 containing 1% FCS. Cell numbers were counted and aliquots (400 μl) of suspended cells were pipetted into coated wells, after which they were cultured for 1 h at 37°C. Non-adherent cells were removed by washing with PBS and attached cells were fixed with 3% paraformaldehyde followed by staining with crystal violet. The number of attached cells was determined visually with a microscope at 100× magnification.

Flow-cytometric analysis Cells were sparsely seeded on 100-mm tissue culture dishes, and cultured in medium containing 1 ng/ml of bFGF and 5% FCS. The next day, cytokines were added to the cultures. After 16 h, cells were harvested nonenzymatically by treatment with 1 m*M* EDTA, and incubated in Hanks' balanced salt solution (HBSS) supplemented with 1% human aggregated IgG, 50 μg/ml of purified goat IgG, 1% bovine serum albumin (BSA), 4% FCS and 0.02% sodium azide for 20 min on ice to block nonspecific binding. They were incubated with the first antibody in HBSS containing 1% BSA and 0.05% sodium azide for 30 min on ice, washed, incubated with the second biotin-conjugated antibody for 20 min, washed, incubated with fluorescein-conjugated avidin (Vector Lab., USA) for 20 min, and analyzed with a FACScan (Becton Dickinson, USA). Antibodies used included rabbit anti-fibroblast growth factor (FGF) receptor/FLG (Upstate Biotechnology, USA), mouse monoclonal anti-human EGF receptor (DAKO), mouse monoclonal anti-fibronectin receptor, which recognizes epitopes in the β_1 integrin subunit (Takara, Kyoto), anti-vitronectin receptor, which recognizes epitopes in the β_3 integrin subunit (Takara), biotin-conjugated goat anti-rabbit IgG (Caltag Lab., USA), and biotin-conjugated goat anti-mouse IgG (Vector Lab.). For the negative control, normal rabbit serum and mouse monoclonal anti-desmin were used.

Angiogenesis assay The assay of FGF-induced angiogenesis was carried out according to the method described by Weckbecker *et al.*[17] with some modifications. Matrix Gel Blue A (Amicon, Tokyo) was washed, diluted with PBS (1:1), and incubated with bFGF for 1 h at room temperature. This suspension (0.1 ml corresponded to 0.5 μg of bFGF) was inoculated into female C57BL/6 mice (14 to 16 g, 4–6 weeks old) intradermally with a 22-gauge needle. Injection sites were on the right and left side at the same level of the body axis. The next day, cytokines were injected at the inoculation sites. Four days after injection of cytokines, the mice were killed, and the number of vessels oriented toward the microspheres was counted under the unified magnification of a dissecting binocular microscope. The vessel counts were done according to the method described by Sidky and Auerbach.[18]

Statistical analysis The statistical significance of differences between groups was calculated by applying one-way analysis of variance and the modified *t* statistics (the Bonferroni method).[19] Data are shown as mean±SD. Synergistic interactions were calculated using an algebraic expression of the isobologram method, as described by Berenbaum.[20] In brief, if the combined agents A and B at the concentrations $[A_{comb}]$ and $[B_{comb}]$ produce exactly the same quantitative effect as the separate agents at the concentrations $[A_s]$ and $[B_s]$, respectively, and

$$\frac{[A_{comb}]}{[A_s]}+\frac{[B_{comb}]}{[B_s]}=1$$

the agents act additively. If the sum of the fraction is less than or greater than 1, the agents act synergistically or

antagonistically, respectively. In the case of no equi-effective concentrations being found, interactions were calculated as in the following example. In Table I, the combination of 10 U/ml of IL-1 and 10 U/ml of IFN-γ caused 75.5% inhibition of cell proliferation of EC. Eqi-effective concentrations of IL-1 and IFN-γ were >100 U/ml, respectively. Substitution of these values into the equation shows that the sum of the fractions $(10/>100)+(10/>100)$ is less than 0.2, so the agents act synergistically.

RESULTS

Effects of IL-1 and/or IFN-γ on EC proliferation EC proliferation was determined by direct cell counting (Table I). Exposure of proliferating EC to IL-1 and IFN-γ led to a dose-dependent decrease in the cell number. At several points, the simultaneous addition of IL-1 and IFN-γ to EC resulted in a similar or a greater inhibition of cell proliferation than that obtained using ten times the amount of each cytokine administered singly, indicating synergism.[20] As shown in Fig. 1, suppression was observed in cells in the logarithmic growth phase and became prominent at 4 days under these culture conditions. In this experiment, IFN-γ alone did not inhibit the proliferation at 6 days, but cotreatment with IL-1 (1 U/ml) and IFN-γ (10 U/ml) suppressed EC proliferation more than IL-1 alone ($P<0.05$). This result also suggests that IL-1 and IFN-γ act in a synergistic manner to decrease EC proliferation. The possible role of endotoxin was ruled out by experiments using polymyxin B to treat cultured EC (not shown). Cytotoxicity was not observed by trypan blue dye exclusion and by measuring the floating cell number of confluent, cultured EC (not shown). These findings were basically consistent with our previous report using mouse aortic EC, in which, however, IL-1 alone did not inhibit EC proliferation.[13]

Effects of IL-1 and/or IFN-γ on EC migration EC migration is another process of angiogenesis. To evaluate cell motility, confluent monolayers were wounded with a plastic pipette tip, and cells were allowed to migrate into the free space for 16 h. As shown in Fig. 2, EC cultured with bFGF (control) actively migrated and filled the wounds. In contrast, EC treated with the combination of IL-1 and IFN-γ were clearly arrested, accompanied with slight elongation of the cell shape. Significant inhibition was observed even by 1 U/ml of IL-1 and 10 U/ml of IFN-γ, whereas each cytokine alone had only a modest effect at such concentrations (Fig. 3). For each individual cytokine to exhibit inhibition of migration, it was necessary to use concentrations tenfold those of the amounts used in combinations. These results indicate that IL-1 and IFN-γ act in a synergistic manner to decrease EC migration.

Effects of IL-1 and/or IFN-γ on EC adhesion Induction of EC adhesion on a matrix is believed to be one of the steps in the process of angiogenesis.[21] We next examined whether these cytokines influence EC adhesion on a matrix. As shown in Fig. 4, EC treated with 1 U/ml or IL-1 and 10 U/ml of IFN-γ showed a significant decrease in adhesion to collagen (69% of control), whereas each cytokine alone at the same dose had no effect. IFN-γ failed to inhibit EC adhesion to culture wells even at 100 U/ml. On the other hand, IL-1 at the concentration of 10

Table I. Inhibition of EC Growth by IL-1 and IFN-γ

IL-1 dose (U/ml)	% of control cell growth (mean±SD)			
	IFN-γ dose (U/ml)			
	0	10	100	1,000
0	100±4.5	89.0±8.3	52.7±1.8	17.0±1.4
1	69.8±6.2	40.0±2.8[a)]	24.5±0.7	
10	52.0±4.3	24.5±10.5[a)]	17.0±1.4[a)]	
100	50.5±4.9			

Representative experiments illustrating the effect of IL-1 and IFN-γ on the proliferation of EC. Cells were incubated with cytokines in the presence of 2 ng/ml of bFGF for 4 days. Results are expressed as % of control growth ([cell number − cell number on day 0/control cell number − cell number on day 0]×100)±SD.
a) Synergistic interaction.

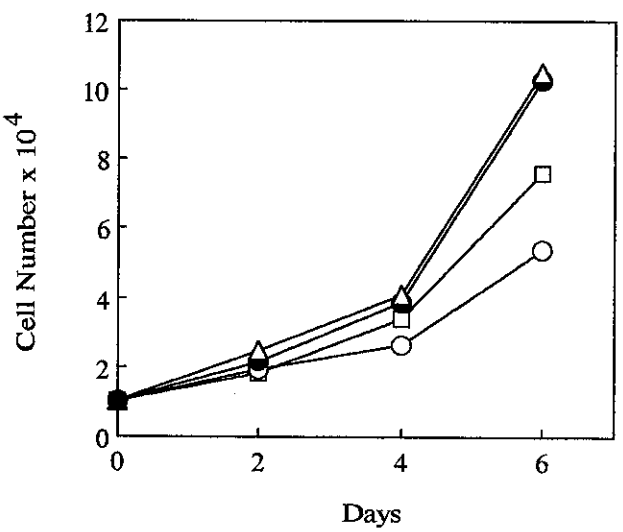

Fig. 1. Kinetics of EC proliferation with cytokines. Cells were treated with cytokines every two days. Cell numbers were counted on the indicated days. Results are expressed as means of triplicate cultures; SDs were less than 10%, Control (●); IL-1 1 U/ml (□); IFN-γ 10 U/ml (△); IL-1 1 U/ml and IFN-γ 10 U/ml (○).

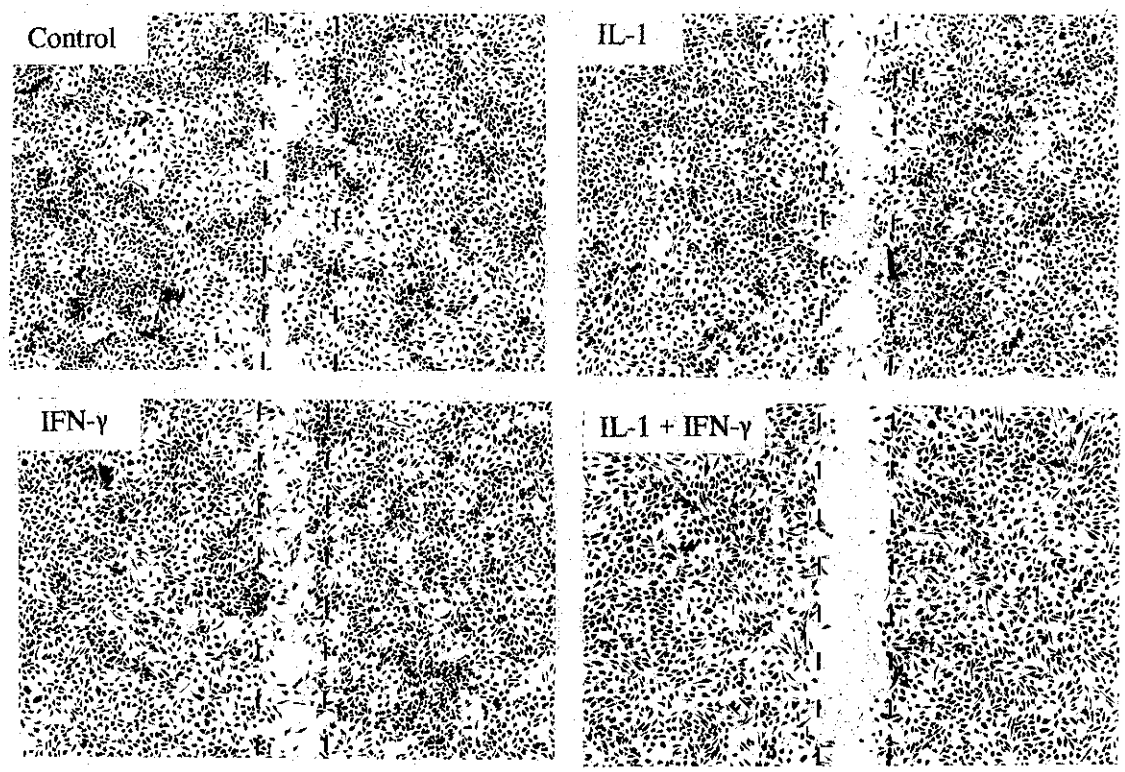

Fig. 2. Effects of IL-1 and/or IFN-γ on EC motility. Monolayers of EC, treated for 48 h in the presence of bFGF (2 ng/ml) with IL-1 (10 U/ml) and/or IFN-γ (100 U/ml), were wounded with a plastic pipette tip and further cultured for 16 h in the presence of the same mediators. The specimens were then fixed, stained, and photographed. The boundary of the wound is indicated by dashed lines.

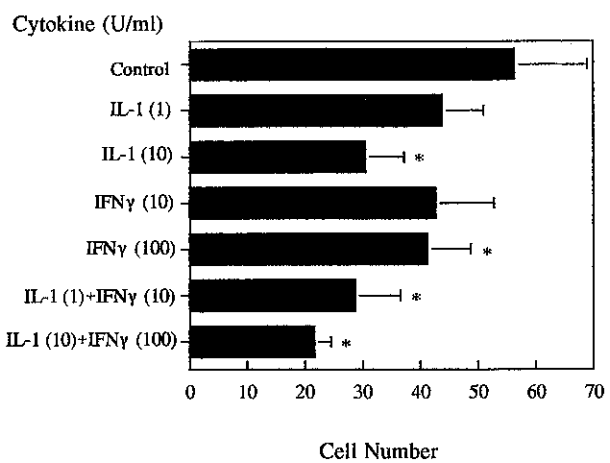

Fig. 3. Quantitative eveluation of EC motility. After treatment of EC with cytokines as described in "Materials and Methods," migration was quantitated by counting the number of cells from the wounded edge using a light microscope. The values represent the mean±SD of nine different fields from triplicate wells. * $P<0.05$ vs. control.

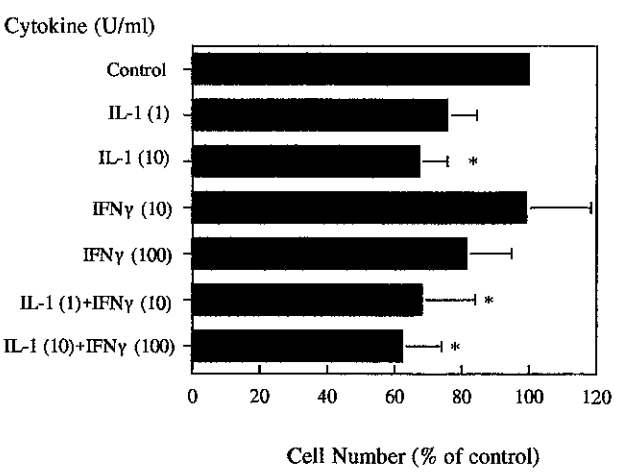

Fig. 4. Effects of IL-1 and/or IFN-γ on EC attachment to collagen. EC, treated for 48 h in the presence of bFGF (2 ng/ml) with cytokines, were trypsinized and then pipetted into collagen-coated wells. After incubation for 1 h at 37°C, cells were fixed and counted. The values are presented as percent of control±SD of four experiments. * $P<0.05$ vs. control.

U/ml inhibited EC adhesion (67% of control). However, these differences between groups remained slight.

IL-1 and/or IFN-γ suppressed FGF receptor expression on EC To determine the mechanism of the angiogenesis-inhibitory activity of these cytokines, the surface receptor expression on EC was investigated. It has been reported that IFN-γ and IL-1 downregulate FGF binding to endothelial cells.[3,8] We examined the expression of FGF receptors as well as EGF receptors on EC by staining with specific antibodies. Integrins of the β_1 and β_3 groups, which are cell surface receptors for extracellular matrix proteins expressed on EC,[15] were also examined. First, analysis of FGF receptors by flow cytometry revealed that the expression of FGF receptors is strongly downregulated by increasing cell density (not shown). Then we cultured EC at low cell density in medium containing 1 ng/ml of bFGF for maximum receptor expression. As shown in Fig. 5, 10 U/ml of IL-1 or 100 U/ml of IFN-γ alone decreased FGF receptor expression on EC. Cotreatments with both cytokines suppressed the FGF receptor expression more markedly. At these concentrations, cytokines suppressed neither the expression of EGF receptors nor β_1 and β_3 integrin subunits.

Effects of IL-1 and/or IFN-γ on bFGF-induced angiogenesis Thus far, these cytokines have been shown to inhibit different processes of angiogenesis *in vitro*. It is important to extend these findings with cultured cells to an *in vivo* angiogenesis model. Angiogenesis was examined by using an bFGF-induced intradermal angiogenesis model, and was evaluated quantitatively. As shown in Fig. 6, bFGF-induced angiogenesis was highly susceptible to the combined treatment with 1,000 U of IL-1 and 100 U of mouse IFN-γ. At this amount, IL-1 stimulated rather than inhibited angiogenesis, and the inhibitory activity of IFN-γ was smaller than that of the combination ($P<0.05$). However, 10,000 U of IL-1 or 1,000 U of mouse IFN-γ totally suppressed bFGF-induced angiogenesis.

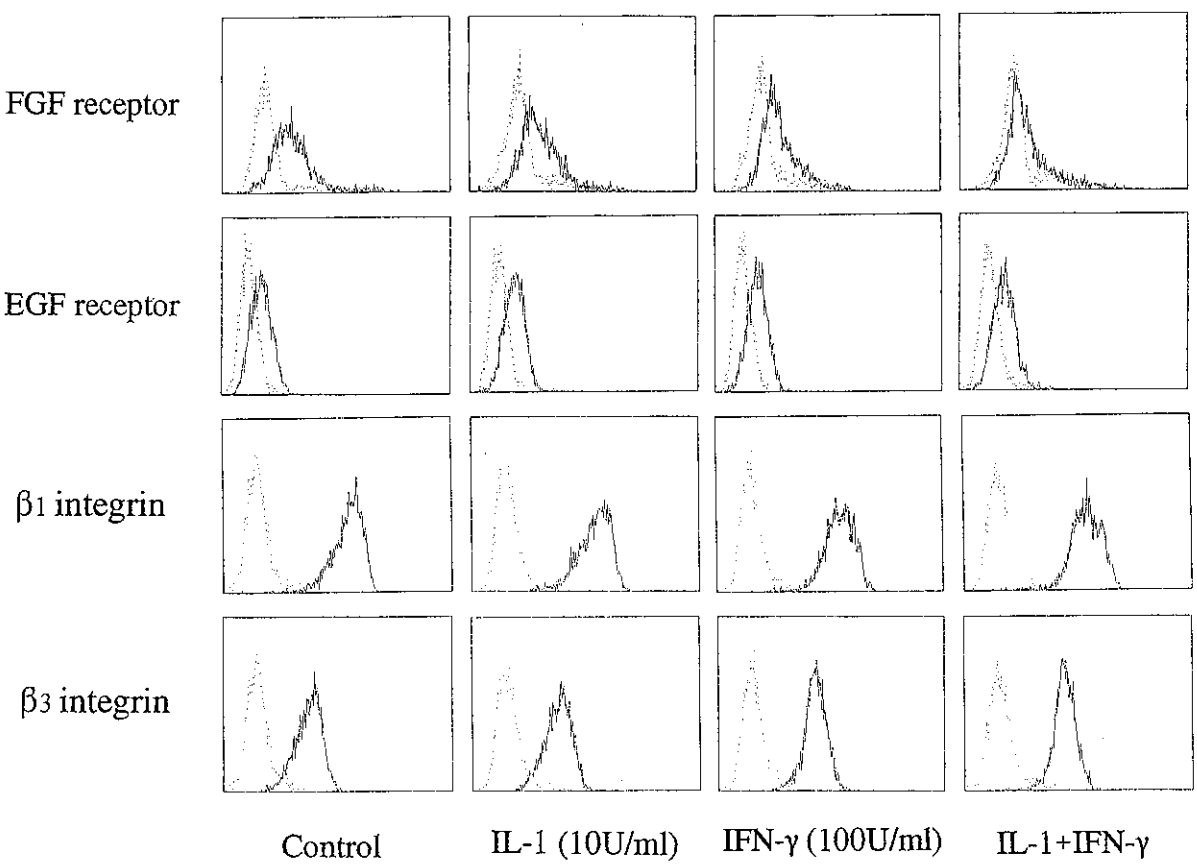

Fig. 5. Effects of IL-1 and/or IFN-γ on receptor expressions on EC. Cells were treated for 16 h with IL-1 (10 U/ml) and/or IFN-γ (100 U/ml). Surface levels of FGF receptors, EGF receptors, β_1 integrin and β_3 integrin were quantitated by indirect immunofluorescence and flow-cytometric analysis. Histograms (with relative fluorescence as a logarithmic scale of 3 log cycles on the x axis, and cell number as a linear scale on the y axis) from one of three experiments are presented. Staining with specific antibodies is shown by solid lines, and staining with nonbinding irrelevant antibodies, by dotted lines.

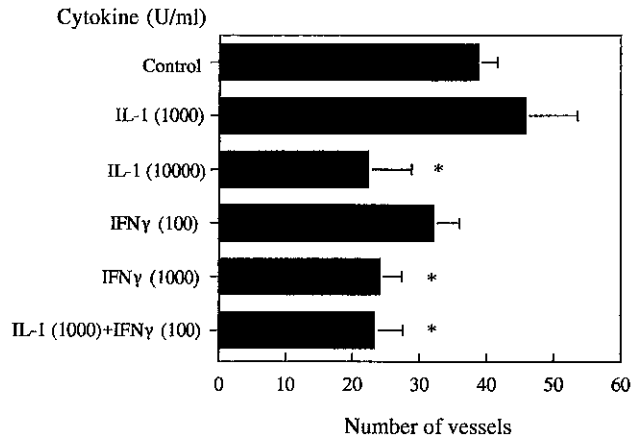

Fig. 6. Effects of treatment with IL-1 and/or mouse IFN-γ injected into the skin of C57BL/6 mice on angiogenesis initiated by bFGF-adsorbed microspheres. Angiogenesis was quantitated by counting the number of vessels oriented toward the mass. Around 17–25 vessels were observed with vehicle (PBS)-adsorbed microspheres. The values represent the mean $\pm$SD of four mice per group. * $P<0.05$ vs. control.

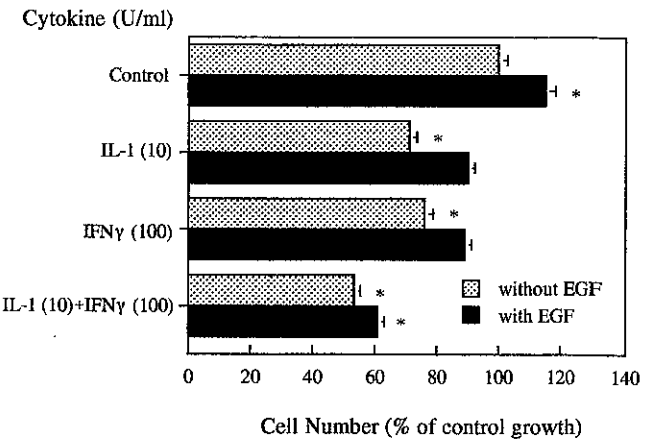

Fig. 7. Effects of EGF on growth of cytokine-treated EC. Cells were incubated with cytokines and 2 ng/ml of bFGF in the absence or presence of 10 ng/ml of EGF for 3 days. Results are expressed as % of control growth ([cell number − cell number on day 0/control cell number − cell number on day 0] $\times$ 100) $\pm$SD of triplicate cultures. * $P<0.05$ vs. control without EGF.

Effects of EGF on proliferation of cytokine-treated EC

The dose of IL-1 required for inhibition of angiogenesis was larger than we had expected. One possible explanation for this is that other growth factors modulate the effects of cytokines *in vivo*. EGF is a mitogen for EC and is present in most tissues.[22] We examined whether EGF could stimulate the proliferation of cytokine-treated EC. As shown in Fig. 7, EGF stimulated EC proliferation, and the inhibitory effects of IL-1 and IFN-γ were partially abrogated. For the cotreated EC, the stimulatory effect of EGF was modest.

DISCUSSION

IL-1 and IFN-γ, individually and synergistically, could inhibit angiogenesis elicited by bFGF in the skin of mice. This synergistic inhibition might be based on inhibition of multiple steps of neovascularization, such as endothelial cell proliferation, migration and adhesion. The expression of FGF receptors was downregulated by these cytokines, whereas those of EGF receptors, integrins β_1 and β_3 were not. EGF could partially restore EC proliferation that was suppressed by each cytokine. These findings suggest that in a situation where FGF plays a major role in angiogenesis, IL-1 and IFN-γ each act as an angiogenesis inhibitor, and the activity can be enhanced by the concomitant presence of both.

Selective downregulation of FGF receptor expression by IL-1 and IFN-γ may be related to the observed inhibitory activities of these cytokines, since FGF stimulates the growth of EC,[22] promotes cell movement,[23] and supports endothelial cell adhesion.[24] However, several other mechanisms could underlie this synergistic inhibition. Montesano *et al.*[25] have reported that cotreatment of EC with IL-1 and IFN-γ rearranged the stress fibers to a form corresponding to the phenotype of stationary, immobile cells. It is possible that the rearranged cytoskeleton of cytokine-treated EC might have affected the proliferation or the migration of EC in our experiments. Another possible mechanism of the inhibition of migration may be decreased protein synthesis of EC following cotreatment with IL-1 and IFN-γ,[26] since protein synthesis is required for the chemotactic migration of fibroblasts.[27] Cell adhesion and migration are also mediated by integrins, which are cell surface receptors for extracellular matrix proteins.[15] However, integrins do not seem to be involved in our study, since the expressions of β_1 and β_3 integrins were not altered by these cytokines (Fig. 5). Roles of growth-inhibiting prostaglandins induced by IL-1, and depletion of L-tryptophan by IFN-γ[28] were ruled out by experiments using cultured EC treated with indomethacin or L-tryptophan (not shown).

Although IL-1 inhibits the proliferation of human umbilical vein EC *in vitro*,[6] IL-1 has recently been demonstrated to stimulate the proliferation of human dermal microvascular EC, and not to inhibit the proliferation of human umbilical vein EC.[29] These findings imply diversity for EC of different origins, but the difference might be due to the different growth factors used in cell culture. In the experiment, both types of EC were

cultured with EGF instead of FGF. Since IL-1 inhibited the expression of FGF receptors but not that of EGF receptors on EC (Fig. 5), the activation of EC by FGF may be a key event for IL-1 to exert its inhibitory action. And this might be applicable to *in vivo* situations. Angiogenesis-inhibitory activity of IL-1 has previously been reported in rabbit cornea stimulated with bFGF.[8] We observed similar inhibitory activity of IL-1 in mouse skin (Fig. 6). In contrast, without FGF, IL-1 has been demonstrated to initiate angiogenesis in rabbit cornea,[9] and mouse skin.[10] Thus, IL-1 may act as a bifunctional angiogenesis regulator which depends on EC activation by FGF.

Utilization of angiogenesis-inhibitory activity by cytokines is expected to be useful therapy for angiogenesis-dependent diseases. However, their therapeutic value has not been established yet. For solid tumors, IFN-γ has been demonstrated to have limited antitumor activity, and it may have an adverse effect in high-risk cutaneous melanoma.[30] Cytokines may only elicit antiangiogenesis activity in limited situations by antagonizing specific angiogenic stimulators, and may not be effective for tumor-induced angiogenesis in which a variety of angiogenesis factors basides FGF are involved.[31, 32] Antiangiogenesis activity elicited by a single agent may thus be of limited value, and its applicability may be limited to the earlier stage of the tumor for effective therapy,[33] and restricted by the other biological actions of cytokines. Therefore, relations between angiogenic stimulators and cytokines as well as means to enhance the specific activities of cytokines should be investigated for therapeutic application. Our studies of cytokines acting synergistically on angiogenesis may provide basic information leading towards clinical use of cytokines as antiangiogenesis agents.

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REFERENCES

1) Folkman, J. and Klagsbrun, M. Angiogenic factors. *Science,* **235,** 442–447 (1987).
2) Folkman, J. Angiogenesis — retrospect and outlook. *In* "Angiogenesis," ed. R. Steiner, P. B. Weisz, and R. Langer, pp. 4–13 (1992). Birkhäuser Verlag, Basel.
3) Friesel, R., Komoriyama, A. and Maciag, T. Inhibition of endothelial cell proliferation by gamma-interferon. *J. Cell Biol.,* **104,** 689–696 (1987).
4) Tsuruoka, N., Sugiyama, M., Tawaragi, Y., Tsujimoto, M., Nishihara, T., Goto, T. and Sato, N. Inhibition of *in vitro* angiogenesis by lymphotoxin and interferon-γ. *Biochem. Biophys. Res. Commun.,* **155,** 429–435 (1988).
5) Sidky, Y. A. and Borden, E. C. Inhibition of angiogenesis by interferons: effects on tumor- and lymphocyte-induced vascular responses. *Cancer Res.,* **47,** 5155–5161 (1987).
6) Norioka, K., Hara, M., Kitani, A., Hirose, T., Hirose, W., Harigai, M., Suzuki, K., Kawakami, M., Tabata, H., Kawagoe, M. and Nakamura, H. Inhibitory effect of human recombinant interleukin-1 α and β on growth of human vascular endothelial cells. *Biochem. Biophys. Res. Commun.,* **145,** 969–975 (1987).
7) Sato, N., Goto, T., Haranaka, K., Satomi, N., Nariuchi, H., Mano-Hirano, Y. and Sawasaki, Y. Actions of tumor necrosis factor on cultured vascular endothelial cells. *J. Natl. Cancer Inst.,* **76,** 1113–1121 (1986).
8) Cozzolino, F., Torcia, M., Aldinucci, D., Ziche, M., Almerigogna, F., Bani, D. and Stern, D. M. Interleukin 1 is an autocrine regulator of human endithelial cell growth. *Proc. Natl. Acad. Sci. USA,* **87,** 6487–6491 (1990).
9) Dunn, C. J., Hardee, M. M. and Staite, N. D. Acute and chronic inflammatory responses to local administration of recombinant IL-1α, IL-1β, TNFα, IL-2 and Ifnγ, in mice. *Agents Actions,* **27,** 290–293 (1989).
10) BenEzra, D., Hemo, H. and Mafzir, G. In vivo angiogenic activity of interleukins. *Arch. Ophthalmol.,* **108,** 573–576 (1990).
11) Fajardo, L. F., Kwan, H. H., Kowalski, J., Prionas, S. D. and Allison, A. C. Dual role of tumor necrosis factor-α in angiogenesis. *Am. J. Pathol.,* **140,** 539–544 (1992).
12) Blood, C. H. and Zetter, B. R. Tumor interactions with the vasculature: angiogenesis and tumor metastasis. *Biochim. Biophys. Acta,* **1032,** 89–118 (1990).
13) Norioka, K., Borden, E. C. and Auerbach, R. Inhibitory effects of cytokines on vascular endothelial cells: synergistic interactions among interferon-γ, tumor necrosis factor-α, and interleukin-1. *J. Immunother.,* **12,** 13–18 (1992).
14) Norioka, K., Hara, M., Harigai, M., Kitani, A., Hirose, T., Suzuki, K., Kawakami, M., Tabata, H., Kawagoe, M. and Nakamura, H. Production of B cell stimulatory factor-2/interleukin-6 activity by human endothelial cells. *Biochem. Biophys. Res. Commun.,* **153,** 1045–1050 (1988).

15) Defilippi, P., Truffa, G., Stefanuto, G., Altruda, F., Silengo, L. and Tarone, G. Tumor necrosis factor α and interferon γ modulate the expression of the vitronectin receptor (integrin β_3) in human endothelial cells. *J. Biol. Chem.*, **266**, 7638–7645 (1991).
16) Asakura, S., Hurley, R. W., Skorstengaard, K., Ohkubo, I. and Mosher, D. F. Inhibition of cell adhesion by high molecular weight kininogen. *J. Cell Biol.*, **116**, 465–476 (1992).
17) Weckbecker, G., Liu, R. and Tolcsvai, L. Intradermal angiogenesis in nude mice induced by human tumor cells or b-FGF. *In* "Angiogenesis," ed. R. Steiner, P. B. Weisz and R. Langer, pp. 296–301 (1992). Birkhäuser Verlag, Basel.
18) Sidky, Y. A. and Auerbach, R. Lymphocyte-induced angiogenesis: a quantitative and sensitive assay of the graft-vs.-host reaction. *J. Exp. Med.*, **141**, 1084–1100 (1975).
19) Wallenstein, S., Zucker, C. L. and Fleiss, J. L. Some statistical methods useful in circulation research. *Circ. Res.*, **47**, 1–9 (1980).
20) Berenbaum, M. C. Criteria for analyzing interactions between biologically active agents. *Adv. Cancer Res.*, **35**, 269–335 (1981).
21) Soncin, F. Angiogenin supports endothelial and fibroblast cell adhesion. *Proc. Natl. Acad. Sci. USA*, **89**, 2232–2236 (1992).
22) Gospodarovicz, D., Brown, K. D., Birdwell, C. R. and Zetter, B. R. Control of proliferation of human vascular endothelial cells. *J. Cell Biol.*, **77**, 774–788 (1978).
23) Moscatelli, D., Presta, M. and Rifkin, D. B. Purification of a factor from human placenta that stimulates capillary cell protease production, DNA synthesis, and migration. *Proc. Natl. Acad. Sci. USA*, **83**, 2091–2095 (1986).
24) Baird, A., Schubert, D., Ling, N. and Guillemin, R. Receptor- and heparin-binding domains of basic fibroblast growth factor. *Proc. Natl. Acad. Sci. USA*, **85**, 2324–2328 (1988).
25) Montesano, R., Orci, L. and Vassalli, P. Human endothelial cell cultures: phenotypic modulation by leukocyte interleukins. *J. Cell Physiol.*, **122**, 424–434 (1985).
26) Doukas, J. and Pober, J. S. IFN-γ enhances endothelial cell activation induced by tumor necrosis factor but not IL-1. *J. Immunol.*, **145**, 1727–1733 (1990).
27) Seppä, H., Grotendorst, G., Seppä, S., Schiffmann, E. and Martin, G. R. Platelet-derived growth factor is chemotactic for fibroblasts. *J. Cell Biol.*, **92**, 584–588 (1982).
28) Takikawa, O., Kuroiwa, T., Yamazaki, F. and Kido, R. Mechanism of interferon-γ action. *J. Biol. Chem.*, **263**, 2041–2048 (1988).
29) Detmar, M., Tenorio, S., Hettmannsperger, U., Ruszczak, Z. and Orfanos, C. E. Cytokine regulation of proliferation and ICAM-1 expression of humann dermal microvascular endothelial cells in vitro. *J. Invest. Dermatol.*, **98**, 147–153 (1992).
30) Meyskens, F. L., Kopecky, K., Samson, M., Hersh, E., Macdonald, J., Jaffe, H., Crowley, J. and Coltman, C. Recombinant human interferon γ: adverse effects in high-risk stage I and II cutaneous malignant melanoma. *J. Natl. Canc. Inst.*, **82**, 1071 (1990).
31) Kandel, J., Bossy-Wetzel, E., Radvanyi, F., Klagsbrun, M., Folkman, J. and Hanahan, D. Neovascularization is associated with a switch to the export of bFGF in the multistep development of fibrosarcoma. *Cell*, **66**, 1095–1104 (1991).
32) Plate, K. H., Breier, G., Weich, H. A. and Risau, W. Vascular endothelial cell growth factor is a potent tumor angiogenesis factor in human gliomas *in vivo*. *Nature*, **359**, 845–848 (1992).
33) Bassukas, I. D., Vester, G. and Maurer-Schultze, B. Cell kinetic studies of endothelial cells in the adenocarcinoma EO771 and the effect of cyclophosphamide. *Virchows Arch. B Cell Pathol.*, **59**, 251–256 (1990).