Interleukin-1β Regulates Angiopoietin-1 Expression in Human Endothelial Cells

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ABSTRACT

Angiopoietin (Ang)-1 is an important regulator of endothelial cell (EC) survival and stabilization. Ang-1 exerts its biological effects by binding to the EC-specific tyrosine kinase receptor Tie-2, and initiates intracellular signaling in ECs. However, regulatory mechanisms for endothelial Ang-1 expression have not been completely elucidated. In this study, we investigated the effects of angiogenic cytokines and growth factors on Ang-1 expression in human umbilical vein ECs (HUVECs). Northern blot analysis was performed after HUVECs were exposed to interleukin-1β (IL-1β), tumor necrosis factor-α, platelet-derived growth factor-BB, insulin-like growth factor-1, or vascular endothelial growth factor (VEGF). Both IL-1β and tumor necrosis factor-α caused marked down-regulation of Ang-1 mRNA levels at 4 h with a further decrease observed at 24 h. Using signaling inhibitors, we identified the P38 pathway as the pathway that mediates IL-1β down-regulation of Ang-1. Furthermore, treatment of cells with IL-1β indirectly (via down-regulation of Ang-1) led to a decrease in Tie-2 autophosphorylation levels in HUVECs. We previously demonstrated that IL-1β regulates VEGF expression in tumor cells. This observation was confirmed in ECs in the present study. Because pericytes play a role in regulating EC function, we also determined whether IL-1β would also down-regulate Ang-1 in human vascular smooth muscle cells. Similar to our findings in HUVECs, we found that IL-1β decreased Ang-1 expression in human vascular smooth muscle cells. Direct effects of IL-1β on angiogenesis were investigated by use of an in vivo Gelfoam angiogenesis assay in which IL-1β produced a significant increase in vessel counts (P = 0.0189). These results suggest that IL-1β indirectly regulates angiogenesis by modulating the expression of Ang-1. IL-1β may trigger a proangiogenic response by decreasing Ang-1 levels in ECs and pericytes and up-regulating VEGF in ECs and tumor cells.

INTRODUCTION

Angiopoietins (Angs) are important regulators of vasculogenesis and postnatal angiogenesis (1–3). Angs mediate their function by interacting with the endothelium-specific tyrosine kinase receptor Tie-2. Ang-1 binding activates the Tie-2 receptor by inducing phosphorylation (4), whereas Ang-2 binds Tie-2 with equal affinity but acts as a naturally occurring antagonist to Ang-1 (5). We and others have demonstrated that overexpression of Ang-1 is associated with angiogenic activity in tumor xenographs in vivo (6, 7). However, little is known about how Ang-1 expression is regulated in endothelial cells (ECs) on exposure to specific angiogenic cytokines and growth factors.

Vascular endothelial growth factor (VEGF) is a potent proangiogenic factor that is frequently overexpressed in a variety of human cancers (8). VEGF expression in tumor cells can be up-regulated by specific cytokines such as interleukin-1β (IL-1β), which has been implicated in the malignant progression and angiogenesis of numerous malignancies (9, 10). VEGF and the Angs seem to play complementary and coordinated roles in the development of new blood vessels (3). IL-1β is a potent immunoregulatory and proinflammatory cytokine secreted by a variety of activated immune cells that can infiltrate solid and tumors (11); it has been shown to be a proangiogenic factor in solid tumors (12). In the present study, we examined the effects of IL-1β on the regulation of Ang-1 and VEGF in ECs. In addition, we investigated the effect of IL-1β on Ang-1 expression in human vascular smooth muscle cells (hvSMCs) as a surrogate for pericytes. IL-1β decreased Ang-1 expression in ECs, and this decrease in expression was mediated via the P38 mitogen-activated protein kinase (MAPK) pathway. In addition, IL-1β down-regulated Ang-1 in hvSMCs, an important observation considering the role the pericytes play in regulating EC function (13). Similar to results observed in previous studies of colon cancer cells and hvSMCs (9, 14), IL-1β led to an increase in VEGF expression. The fact that IL-1β can up-regulate VEGF and down-regulate Ang-1 suggests that IL-1β may be an important indirect regulator of tumor angiogenesis.

MATERIALS AND METHODS

Cell Culture Conditions. Human umbilical vein ECs (HUVECs) and hvSMCs were purchased from the American Type Culture Collection (Manassas, VA). HUVECs were cultured in gelatin-coated (0.5%) culture flasks in MEM supplemented with 15% fetal bovine serum (FBS), basic fibroblast growth factor (10 ng/ml), insulin, Purchase, NY), vitamins, penicillin–streptomycin, sodium pyruvate, I-glutamine, and nonessential amino acids; hvSMCs were cultured in Hanks’ modified DMEM supplemented with 10% FBS at 37°C in 5% CO2 and 95% air. For all in vitro experiments, cells were grown to 80–90% confluence and incubated under serum-reduced conditions (5% FBS-MEM) overnight before the experiments were conducted. Results from all studies were confirmed in at least three independent experiments.

RNA Isolation and Northern Blot Analysis. For Northern blot analyses, total RNA was extracted from cells by TRizol (Life Technologies, Inc.) according to the manufacturer’s instructions. Northern blot analysis was performed as described previously (6). Briefly, probes for Ang-1 (a 535-bp fragment of Ang-1 was a gift from Tona Gilmer, GlaxoSmithKline Inc., Research Triangle Park, NC), VEGF (a 204-bp cDNA probe was a gift from Dr. Brygida Berse, Harvard Medical School, Boston, MA), or for glyceraldehyde-3-phosphate dehydrogenase (Amersham Biosciences) were subsequently hybridized overnight before the experiments were conducted. Autoradiography was performed thereafter in the linear range of the film (Hyperfilm MP; Amersham Biosciences). For in vitro experiments investigating cytokine-dependent Ang-1 mRNA expression, cells were treated with insulin-like growth factor-1 (100 ng/ml), platelet-derived growth factor-BB (10 ng/ml), IL-1β (10 ng/ml), tumor necrosis factor-α (TNF-α; 10 ng/ml), or VEGF (10 ng/ml; all cytokines were purchased from R&D Systems Inc., Minneapolis, MN). Studies to identify relevant signaling pathways for IL-1β-regulated Ang-1 expression in HUVECs were performed with specific inhibitors to extracellular signal-regulated kinase 1/2 (Erk-1/2; 50 μM UO126; New Eng-land Biolabs Inc., Beverly, MA), P38 MAPK (10 μM SB203580; Calbiochem, San Diego, CA), or phosphatidylinositol 3-kinase/Akt (200 nM Wortmannin; Sigma, St. Louis, MO). HUVECs were treated with individual inhibitors for 1 h in 1% FBS-MEM before the addition of IL-1β (10 ng/ml). Doses for each

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signaling inhibitor were previously tested for their toxicity to HUVECs to ensure cell viability throughout the experiments.

Western Blot Analysis. Total and phosphorylated protein levels were determined by Western blot analyses as described previously (14). Briefly, protein was extracted from cell lysates by use of RIPA buffer, and 40-μg protein samples were subjected to Western blot analysis on denaturing 6% or 10% SDS-PAGE gels (15). Activated signaling pathways were identified by use of the following antibodies (all from Cell Signaling Technology, Beverly, MA): anti-Erk-1/2, anti-phosphospecific Erk-1/2 [Phospho-p44/42 MAPK (Thr202/Tyr204)], anti-Akt, anti-phosphospecific Akt (Phospho-Akt Ser473), anti-phosphospecific c-jun-terminal kinase/stress-activated protein kinase [Phospho-SAPK/JNK (Thr183/Tyr185)], anti-P38, and anti-phosphospecific P38 [Phospho-p38 MAPK (Thr180/Tyr182)].

Quantification of VEGF Protein in Conditioned Medium (CM) from HUVECs Treated with IL-1β. CM from HUVECs was prepared as follows. Cells were grown to 80–90% confluence and were incubated for 48 h in 1% FBS with IL-1β (10 ng/ml; “activated” CM) or without IL-1β (control CM). The CM was collected and centrifuged, followed by filtration through Amicon Centriprep filters (PM10) according to the manufacturer’s protocol (Millipore, Bedford, MA). The CM was quantitated spectrophotometrically by the BCA (Pierce) assay. VEGF ELISA was performed according to the manufacturer’s protocol (R&D Systems).

Immunoprecipitation of Ang-1 or Phosphorylated Tie-2. HUVECs were incubated for 24 or 48 h in the presence of 1% IL-1β (10 ng/ml) in 1% FBS-MEM. Protein was extracted as described above. Aliquots (500 μg) of protein samples were immunoprecipitated with goat antihuman Ang-1 antibody or rabbit antihuman Tie-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) by overnight rotation at 4°C in RIPA buffer containing agarose beads (Ag Plus Agarose; Santa Cruz Biotechnology). The beads were washed three times with cold RIPA buffer and resolved on denaturing 10% (for Ang-1) or 6% (for Tie-2) SDS-PAGE gels as described above. Western blot membranes were probed with mouse antihuman Ang-1 antibody (R&D Systems) or mouse antiphosphotyrosine antibody (Upstate Biotechnology, Lake Placid, NY). For Tie-2, equal loading was verified by reprobing the membrane with Tie-2 antibody. For immunoprecipitation studies, a nonspecific IgG antibody was used to confirm the absence of nonspecific binding.

Gelfoam in Vivo Angiogenesis Assay. Effects of IL-1β on angiogenesis were investigated in a Gelfoam in vivo angiogenesis assay using male BALB/c mice. Sterile absorbable sponges (Pharmacia, Peapack, NJ) were cut into 7-mm pieces and hydrated overnight at 4°C in sterile PBS. Excess PBS was then drained by blotting on sterile filter paper. The sponges were then soaked in 0.4% agarose (100 μl) containing either PBS (control) or IL-1β (1 μg/ml). The agarose-Gelfoam plugs were then allowed to harden for 1 h at room temperature before being implanted s.c. into BALB/c mice (five mice/group). Mice were anesthetized with Nembutal (50 mg/kg i.p.), and the plugs were implanted s.c. via a midline incision of the abdominal skin; one plug was placed s.c. at least 2 cm away from the incision site on either side. The wound was closed with surgical metal clips. Fourteen days later, mice were sacrificed. The Gelfoam plugs were removed and washed once in PBS and then frozen in OCT compound. Specimens were snap-frozen in liquid nitrogen for subsequent immunohistochemical analysis.

Immunohistochemical Analyses of Vessel Density. Rat antimonoclonal CD31/PECAM-1 antibody was obtained from Pharmingen (San Diego, CA) and peroxidase-conjugated goat antirat IgG from Jackson Research Laboratories (West Grove, PA). Gelfoam plugs that had been frozen in OCT were sectioned in 20-μm slices, mounted on positively charged slides, and air-dried for 30 min. Tissue sections were then fixed in cold acetone followed by 1:1 acetone–ethanol and acetonized for 5 min, then washed with PBS. Specimens were then incubated with 3% H2O2 in methanol for 12 min at room temperature to block endogenous peroxidase, washed three times with PBS (pH 7.5), and incubated for 20 min at room temperature in a protein-blocking solution consisting of PBS supplemented with 1% normal goat serum and 5% normal horse serum. The primary antibody directed against CD31 was diluted 1:800 in protein-blocking solution and applied to the sections, which were then incubated overnight at 4°C. Sections were then rinsed in PBS and incubated for 10 min in protein-blocking solution before the addition of peroxidase-conjugated secondary antibody. The secondary antibody used for CD31 (peroxidase-conjugated goat antirat IgG) was diluted 1:200 in protein-blocking solution. After incubation with the secondary antibody for 1 h at room temperature, the samples were washed and incubated with stable diaminobenzidine (Research Genetics, Huntsville, AL) substrate. Staining was monitored under a bright-field microscope, and the reaction was stopped by washing with distilled water. Sections were mounted with Universal Mount (Research Genetics). CD31-stained vessels were counted (at ×100 magnification) in five different quadrants of each Gelfoam plug (2 mm inside the edge), and averages were calculated. For all immunohistochemical studies, the primary antibody was omitted as a negative control.

Analysis of Immunostained Tissue Sections. The images were captured and analyzed by Optimas image analysis software (version 5.2; Bothell, WA). Positive cells were counted by NIH Image Analysis software (version 1.62) from the NIH (Bethesda, MD). The number of positive cells was expressed as the average of the number of positive staining cells per high-power field at ×100. Five fields from one 20-μm-thick section/specimen were chosen randomly, and five mice/group underwent analysis.

Statistical Analyses. All statistical analyses were done with InStat Statistical Software (version 2.03; GraphPad Software, San Diego, CA); with P < 0.05 considered statistically significant.

Densitometric Quantification. Densitometric analysis of autoradiographs was performed with NIH Image Analysis software (version 1.62) from the NIH to quantify the results of Northern and Western blot analyses.

RESULTS

Regulation of Ang-1 Expression in ECs by Cytokines and Growth Factors. To investigate the effects of various cytokines and growth factors associated with angiogenesis on endothelial Ang-1 expression, we treated HUVECs with insulin-like growth factor-1, platelet-derived growth factor-BB, IL-1β, TNF-α, or VEGF for 4 and 24 h. Northern blot analysis revealed that treatment with IL-1β or TNF-α led to marked decreases in Ang-1 mRNA expression at 4 h, with further decreases evident at 24 h. At 24 h, Ang-1 mRNA expression was decreased 70% by IL-1β and 90% by TNF-α (Fig. 1A). Similarly, Ang-1 protein expression was decreased at 24 h, as determined by immunoprecipitation (Fig. 1B). To confirm these ob-
colon cancer cells and hVSMCs (9, 14), we found that IL-1β expression in endothelial cells. Human umbilical vascular endothelial cells were stimulated with IL-1β (10 ng/ml) in MEM containing 1% fetal bovine serum for the indicated times, and changes in VEGF mRNA expression were investigated by Northern blot analysis. Maximum effects of IL-1β on VEGF expression were observed at 24 h (~5-fold increase compared with 24-h control). VEGF protein levels were determined by ELISA after stimulation with IL-1β for 48 h. IL-1β led to a ~4-fold increased in VEGF protein levels compared with control (b). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Fig. 2. Interleukin-1β (IL-1β) up-regulates vascular endothelial growth factor (VEGF) expression in endothelial cells. Human umbilical vascular endothelial cells were stimulated with IL-1β (10 ng/ml) in MEM containing 1% fetal bovine serum for the indicated times, and changes in VEGF mRNA expression were investigated by Northern blot analysis. Maximum effects of IL-1β on VEGF expression were observed at 24 h (~5-fold increase compared with 24-h control). VEGF protein levels were determined by ELISA after stimulation with IL-1β for 48 h. IL-1β led to a ~4-fold increased in VEGF protein levels compared with control (b). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Effect of IL-1β expression in ECs. A, time course of down-regulation of Ang-1 by IL-1β. Human umbilical vascular endothelial cells were stimulated with IL-1β (10 ng/ml) in MEM containing 1% fetal bovine serum for the indicated times, and changes in Ang-1 mRNA expression were investigated by Northern blot analysis. Maximum effects of IL-1β stimulation were detectable at 24 h (70% decrease). B, effect of various concentrations of IL-1β on Ang-1 expression. At 0.01 ng/ml, IL-1β reduced Ang-1 mRNA levels by 40%; a 70% reduction was seen at higher concentrations. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Effect of IL-1β on VEGF Expression in HUVECs. Because VEGF and Ang-1 play complementary and coordinated roles in vascular development and tumor angiogenesis, we hypothesized that IL-1β would increase VEGF in HUVECs. Similar to our findings in colon cancer cells and hVSMCs (9, 14), we found that IL-1β produced to an ~3-fold increase in VEGF mRNA expression at 4 h and an ~5-fold increase in VEGF mRNA expression at 24 h as determined by Northern blot (Fig. 2A). Similar to VEGF mRNA levels, we found that IL-1β produced an ~4-fold increase in VEGF protein expression at 48 h as determined by ELISA (Fig. 2B).

Effect of IL-1β on Ang-1 Expression in ECs. We treated HUVECs with IL-1β for 1, 3, 6, and 24 h to determine the kinetics of the decrease in Ang-1 produced by IL-1β treatment. Northern blot analysis demonstrated a time-dependent decrease in Ang-1 mRNA expression after incubation with IL-1β, with the maximum decrease (~70% compared with controls) at 24 h (Fig. 3A). This time point was selected for use in subsequent studies. We also investigated the effects of IL-1β concentration on Ang-1 regulation by treating HUVECs with increasing doses of IL-1β (0.01–10 ng/ml). Decreases in Ang-1 mRNA levels were detectable after IL-1β treatment starting at a dose of 0.01 ng/ml, resulting in a ~40% decrease in Ang-1 mRNA expression compared with control cells (Fig. 3B). Increasing the IL-1β concentration to 10 ng/ml produced an additional down-regulation of Ang-1 expression (~70%). Repeat studies verified the above results.

Effect of IL-1β on Intracellular Signaling Pathway Activation. To identify the signaling intermediates activated by IL-1β, we incubated HUVECs with or without IL-1β (10 ng/ml) in 1% MEM-FBS for various times. Western blot analysis showed that IL-1β treatment increased the phosphorylation levels of Akt, Erk 1/2, and P38 in a time-dependent manner starting at 10 min (Fig. 4A). No changes were detected in JNK phosphorylation (data not shown). To identify the relevant signaling pathways involved in the IL-1β-mediated down-regulation of Ang-1 mRNA, we incubated HUVECs with inhibitors to phosphatidylinositol 3’-kinase/Akt (Wortmannin), Erk 1/2 (UO126), or P38 (SB203580), followed by a 24-h incubation with IL-1β. Northern blot analysis revealed that inhibition of P38 activation blunted the IL-1β-mediated down-regulation of Ang-1 mRNA (Fig. 4B). In contrast, the MAPK and phosphatidylinositol 3’-kinase inhibitors had minimal effects.

Regulation of Tie-2 Receptor Tyrosine Phosphorylation by IL-1β. We investigated the molecular significance of the IL-1β-mediated down-regulation of Ang-1 by comparing Tie-2 phosphorylation levels in treated versus untreated HUVECs. In these assays, cells were treated with IL-1β for 24 and 48 h, and protein was extracted at each time point for subsequent immunoprecipitation of Tie-2 and Western blot analysis of phosphotyrosine. IL-1β-mediated down-regulation of Ang-1 led to a significant (30–40%) reduction in Tie-2 autophosphorylation levels in treated cells compared with control cells at 24 and 48 h (Fig. 5). Repeat studies verified the above results.

Effect of IL-1β on Angiogenesis. To demonstrate that IL-1β may be an important regulator of angiogenesis, we studied the effects of IL-1β on angiogenesis in vivo. For this purpose, we used a Gelfoam in vivo angiogenesis assay in which agarose-Gelfoam sponges were implanted subcutaneously into male C3H mice (9-12 weeks old). Sponges were cut into large pieces and implanted subcutaneously into mice. IL-1β (100 ng/ml) was applied to the sponges at the time of implantation. After 7 days, the sponges were removed, and the capillary length was measured. As shown in Fig. 6, IL-1β significantly reduced the capillary length compared with control sponges. These results suggest that IL-1β may be a potential target for the treatment of angiogenesis-related diseases.

Fig. 3. Interleukin-1β (IL-1β) reduces angiopoietin-1 (Ang-1) expression in endothelial cells. A, time course of down-regulation of Ang-1 by IL-1β. Human umbilical vascular endothelial cells were stimulated with IL-1β (10 ng/ml) in MEM containing 1% fetal bovine serum for the indicated times, and changes in Ang-1 mRNA expression were investigated by Northern blot analysis. Maximum effects of IL-1β stimulation were detectable at 24 h (70% decrease). B, effect of various concentrations of IL-1β on Ang-1 expression. At 0.01 ng/ml, IL-1β reduced Ang-1 mRNA levels by 40%; a 70% reduction was seen at higher concentrations. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Ang-1 by IL-1 

analysis for Ang-1 mRNA was performed after 24 h incubation. Down-regulation of cells. Human umbilical vascular endothelial cells were incubated with or without IL-1 (P/H11005 the controls at each time point. This study was repeated with similar results. IL-1 and P38.

Fig. 4. Effects of interleukin-1 (IL-1) on signaling pathway activation in endothelial cells. Human umbilical vascular endothelial cells were incubated with or without IL-1β (10 ng/ml) for the indicated times, and protein was extracted. A. Western blot analyses for total and phosphorylated forms of Akt, extracellular signal-regulated kinase 1/2 (Erk 1/2), and P38. B, effect of IL-1β stimulation, in combination with specific signaling inhibitors of phosphatidylinositol 3′-kinase/Akt (Wortmannin), Erk 1/2 (UO126), or P38 (SB203580) on angiopoietin-1 (Ang-1) expression in endothelial cells. Northern blot analysis for Ang-1 mRNA was performed after 24 h incubation. Down-regulation of Ang-1 by IL-1β was mediated by the P38 pathway. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Fig. 5. Interleukin-1β (IL-1β) decreases Tie-2 autophosphorylation in endothelial cells. Human umbilical vascular endothelial cells were incubated in MEM containing 1% fetal bovine serum with or without IL-1β (10 ng/ml) for 24 or 48 h, and protein was extracted for subsequent Tie-2 immunoprecipitation and Western blot analysis. By densitometry, IL-1β treatment decreased Tie-2 autophosphorylation levels by 30–40% with respect to the controls at each time point. This study was repeated with similar results.

soaked with either IL-1β (1.0 μg/mL) or PBS alone (control) and implanted s.c. in mice. Significantly more microvessels were present in the IL-1β-soaked Gelfoam plugs than in the PBS control plugs (P = 0.0189; Fig. 6).

DISCUSSION

In this study, we demonstrated that both IL-1β and TNF-α decreased Ang-1 mRNA expression in human ECs and that the effects of IL-1β were selectively mediated via the P38 MAPK signaling pathway. Considering the role of IL-1β as a proangiogenic cytokine (9, 16, 17), we focused on elucidating its effects on the endothelial expression of Ang-1, which regulates EC survival and exerts antiangiogenic properties in vivo (2, 6, 18, 19). Although it has previously been shown that IL-1β and TNF-α decrease Ang-1 expression in HUVECs (20), our study has expanded these observations to demonstrate the mechanism by which this occurs. We have also demonstrated that the reduction of Ang-1 in ECs leads to a decrease in the autocrine activation of Tie-2.

IL-1β has been implicated in colon cancer angiogenesis because of its ability to up-regulate VEGF expression in HT29 colon cancer cells in vitro (9). We previously demonstrated that exposure of HT29 cells to IL-1β caused a >5-fold increase in VEGF mRNA expression at 24 h that was associated with increases in VEGF promoter activity and protein expression (9). Furthermore, IL-1β may enhance tumor angiogenesis by promoting the induction of VEGF by other cell types within the tumor microenvironment. Small tumor vessels are composed of ECs and pericytes (or derivatives of VSMCs) that communicate with each other via cytokine signaling during the angiogenic process. We recently investigated the effects IL-1β on VSMCs, showing that IL-1β increased VEGF expression in VSMCs (14). Data from this previous study suggested that IL-1β may mediate EC survival and angiogenesis by induction of VEGF in a paracrine manner (14). This regulation of VEGF expression in VSMCs was mediated by the P38 MAPK signaling pathway (14). In the present study, we were able to demonstrate that the proangiogenic molecule IL-1β can also down-regulate endogenous inhibitors of angiogenesis, such as Ang-1, in ECs and that this mechanism was mediated via the P38 MAPK pathway. Several cytokines have been shown to activate the P38 signaling pathway in ECs, and in general, P38 activation has been associated with decreased EC survival (21). However, down-regulation of Ang-1, an important EC survival factor, by IL-1β may play an additional role in the mechanism for P38-mediated decrease in cell survival.

Fig. 6. Effect of interleukin-1β (IL-1β) on vessel density in an in vivo angiogenesis assay. Agarose-Gelfoam sponges containing either IL-1β (1.0 μg/mL) or PBS (control) were implanted s.c. into mice, where they remained for 14 days before being harvested, sectioned, and stained. CD31 staining revealed more vessels in the IL-1β group than in the control group (P = 0.0189). HPF, high-power field.
Ang-1 is a secreted growth factor that activates the Tie-2 receptor tyrosine kinase, which enhances EC survival and capillary morphogenesis and also limits capillary permeability. Ang-1 is expressed by ECs, smooth muscle cells, pericytes, and their precursors and exerts paracrine effects on Tie-2-expressing ECs (1, 4, 5). In the present study, we found that IL-1β-mediated down-regulation of Ang-1 led to a significant reduction in Tie-2 phosphorylation in ECs. In contrast to the findings of others, where Tie-2 protein levels were slightly increased after cytokine treatment (22), we did not observe any changes in total Tie-2 protein levels.

Another cytokine associated with angiogenesis and tumor growth is TNF-α, which may also induce the expression of VEGF and other proangiogenic factors in cells (23). In the present study, treatment of HUVECs with TNF-α, like IL-1β, resulted in a marked reduction in endogenous Ang-1 mRNA expression. We chose to investigate IL-1β rather than TNF-α because IL-1β inhibitors are already in clinical use for arthritis; the antiangiogenic effect of these agents may, in part, explain their efficacy (24).

Regulation of cellular Ang-1 expression has important implications in angiogenesis given that Ang-1 exerts antiangiogenic activity in vivo and stabilizes ECs in vitro (4, 6, 7). We and others have shown that stable overexpression of Ang-1 in various tumor systems inhibits tumor angiogenesis, tumor growth, and vascular permeability (6, 7, 18, 19).

In summary, our results demonstrate that IL-β can down-regulate the expression of Ang-1 and that this occurs concomitant to an increase in VEGF expression in HUVECs. This mechanism results in a net gain of proangiogenic stimuli that overall may promote and potentiate neovascularization in tumors. Inhibition of IL-1β-mediated effects may therefore be a valuable approach for antiangiogenic regimens to decrease VEGF expression in multiple cell types within tumors and maintain Ang-1 levels in ECs and pericytes.

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