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Interleukin 1 regulates heparin-binding growth factor 2 gene expression in vascular smooth muscle cells

(basic fibroblast growth factor/interferon γ/glucocorticoids/atherosclerosis)

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ABSTRACT **The angiogenic polypeptide heparin-binding growth factor 2 (HBGF-2), or basic fibroblast growth factor, is a mitogen for vascular smooth muscle cells *in vitro* and *in vivo*. Smooth muscle cells also synthesize HBGF-2; thus, it may stimulate their proliferation *in vivo* by both autocrine and paracrine mechanisms. We report here that HBGF-2 gene expression in human saphenous vein smooth muscle cells is induced by interleukin (IL)-1α and IL-1β, inflammatory cytokines produced by many cell types in response to a variety of signals. Maximal HBGF-2 mRNA levels are detected 2–4 hr after IL-1 treatment; induction may require *de novo* protein synthesis and does not occur if transcription is inhibited. Immunoprecipitation analysis indicates that IL-1-stimulated cells also express an increased amount of HBGF-2 protein. Interferon γ and glucocorticoids, inhibitors of smooth muscle cell proliferation *in vitro* and *in vivo*, suppress the induction of HBGF-2 expression by IL-1. These results imply that cytokines released at sites of vascular injury or inflammation may regulate HBGF-2 production by smooth muscle cells. Increased HBGF-2 levels within the vessel wall could play a role in both the smooth muscle cell proliferation and the neovascularization associated with the development of atherosclerotic lesions.**

Heparin-binding growth factor (HBGF)-1 and HBGF-2, also commonly characterized as acidic and basic fibroblast growth factor, respectively, are structurally related members of the HBGF family of proteins (reviewed in refs. 1 and 2). Numerous biological properties of HBGF-1 and HBGF-2 indicate that they could play an important role in the pathogenesis of certain vascular diseases (reviewed in ref. 3). For example, smooth muscle cell migration and proliferation occurs during atherogenesis (reviewed in ref. 4). HBGF-1 is a chemotactic factor (5) and both polypeptides are mitogenic (6) for smooth muscle cells cultured *in vitro*. Also, the administration of HBGF-2 after balloon catheter injury of the rat carotid artery induces smooth muscle cell proliferation and intimal thickening (7). Another process in which the two HBGFs may be involved is the neovascularization of atherosclerotic plaques (8). Both polypeptides stimulate endothelial cell proliferation and migration *in vitro* (9–11) and induce angiogenesis *in vivo* (9, 12).

Vascular endothelial and smooth muscle cells not only respond to HBGF-1 and HBGF-2 but may be sources for these polypeptides *in vivo*. Human and bovine endothelial and smooth muscle cells cultured *in vitro* express HBGF-2 (13–16). HBGF-1 mRNA is present in both human endothelial and smooth muscle cells, but the expression level is significantly higher in smooth muscle cells (refs. 16 and 17; unpublished data). HBGF-1 and HBGF-2 are also expressed by vascular cells *in vivo*. The mRNAs encoding both HBGFs are present in rat aortic tissue (18); in addition, immunoreactive HBGF-2 protein has been detected in medial smooth muscle cells of rat arteries (19). Although these results suggest that vessel wall-derived HBGF-1 and HBGF-2 could stimulate vascular cell proliferation by both autocrine and paracrine mechanisms, it should be noted that neither protein contains a hydrophobic signal peptide required for secretion via the classical secretory pathway. Therefore, they may not normally exit the cell; in this case, autocrine effects could only occur if they act intracellularly or if they were released as a result of sublethal cell damage. Paracrine stimulation of neighboring cells could only occur if the HBGFs were released via cell injury or lysis. Alternatively, the two HBGFs may be actively released from cells by an unknown secretory mechanism. This possibility is supported by studies demonstrating that HBGF-2-specific neutralizing antibodies inhibit the basal growth (20–22) and migration (20) of bovine endothelial cells.

As an approach to identify factors that may modulate HBGF-1 and HBGF-2 production by vascular cells *in vivo*, we are investigating the regulation of HBGF gene expression by using smooth muscle cells cultured *in vitro*. Previous studies have indicated that HBGF-2 mRNA levels increase after serum stimulation of quiescent human foreskin fibroblasts (23) and after phorbol ester treatment of human umbilical vein endothelial cells (24). Serum and phorbol ester also induce HBGF-2 gene expression in human astrocytoma cells (25, 26). In this report, we demonstrate that the multifunctional cytokine interleukin (IL)-1 can induce HBGF-2 gene expression in human vascular smooth muscle cells and skin fibroblasts.

MATERIALS AND METHODS

Cell Culture. Human aortic and saphenous vein smooth muscle cells were generously supplied by P. Libby (Harvard Medical School, Boston). They were grown on fibronectin-coated flasks in Dulbecco's modified Eagle's medium (low glucose, buffered with 25 mM Hepes; Whittaker Bioproducts) supplemented with 10% fetal bovine serum (HyClone), 2 mM L-glutamine (GIBCO), and 1× antibiotic/antimycotic (GIBCO). To induce quiescence, these cells were incubated for 72 hr in the medium described above containing 0.5% serum. The human saphenous vein smooth muscle cells (HSVSMCs) were used for most of the experiments described here. They were routinely expanded by trypsin treatment and subculturing at a 1:3 split ratio. All experiments used cells between passage levels 5 and 8. Human skin

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Abbreviations: HBGF, heparin-binding growth factor; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; HSVSMC, human saphenous vein smooth muscle cell; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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fibroblasts (GM 2037; Human Genetic Mutant Cell Repository, Camden, NJ) were grown to confluence in Dulbecco's modified Eagle's medium (high glucose, with glutamine; Mediatech), 10% fetal bovine serum, 1× antibiotic/antimycotic and were rendered quiescent by incubation for 72 hr in the medium described above containing 0.5% serum. Human umbilical vein endothelial cells (a gift of M. Gimbrone, Harvard Medical School) were grown in HBGF-1-containing medium as described (27) and were rendered quiescent by incubation for 24 hr in 0.5% serum-containing medium without HBGF-1.

RNA Gel Blot Analysis. For the experiment reported in Fig. 1*A*, quiescent cells were treated with recombinant human IL-1α (10 ng/ml), IL-1β (10 ng/ml), IL-6 (40 ng/ml), or tumor necrosis factor (TNF)-α (20 ng/ml), which were obtained from Genzyme. For all additional experiments, cells were treated with recombinant human IL-1α kindly provided by P. Lomedico (Hoffmann–La Roche). In the IL-1α antibody neutralization experiments, cells were treated for 4 hr with IL-1α (1 ng/ml) that had been incubated for 16 hr at 4°C in either phosphate-buffered saline (Mediatech), phosphate-buffered saline containing 1 mg of nonimmune rabbit IgG (Sigma), or phosphate-buffered saline containing 1 mg of rabbit anti-human IL-1α polyclonal antibody (Genzyme). Cycloheximide (10 μg/ml; Sigma), actinomycin D (2 μg/ml; Calbiochem), dexamethasone (2 μM; Sigma), hydrocortisone (200 μM; Sigma), and recombinant human interferon (IFN)-γ (50 ng/ml; Boehringer Mannheim) were also used for some RNA gel blot experiments. Cells were harvested, total RNA was prepared as described (17), and 10 μg of each sample was denatured and subjected to electrophoresis in 1.2% agarose gels containing 2.2 M formaldehyde. The gels were stained with ethidium bromide to verify that each lane contained similar amounts of undegraded ribosomal RNA. RNA was electroblotted onto Zetabind nylon membranes (AMF Cuno) and cross-linked by UV irradiation using a Stratalinker (Stratagene). The restriction fragments used and the source of DNA probes were as follows: (*i*) bovine HBGF-2, 1.4-kilobase (kb) *Eco*RI fragment of pJJ11-1 (gift of J. Abraham, California Biotechnology, Mountain View, CA); (*ii*) human IL-1β, 0.7-kb *Eco*RI/*Nde* I fragment of pΔ11B (gift of U. Gubler, Hoffmann–La Roche); (*iii*) human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 0.8-kb *Pst* I/*Xba* I fragment of pHcGAP (American Type Culture Collection). The probes were labeled to high specific activity with [^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) using a random primer labeling kit (Boehringer Mannheim). Hybridization and membrane washing conditions were as described (27). Blots were exposed to film (XAR-5; Kodak) with an intensifying screen at −80°C. Autoradiographic signals were quantitated with an LKB laser densitometer; the degree of HBGF-2 mRNA induction was calculated after normalization to GAPDH mRNA levels.

Immunoprecipitation Analysis. HSVSMCs were incubated for 72 hr in standard medium containing 0.5% fetal bovine serum, and an equivalent number of cells either were left untreated or were treated with IL-1α (10 ng/ml) for 2 hr. The cells were rinsed twice with serum-free/cysteine-free medium and then incubated for 6.5 hr in this medium with or without IL-1α. The medium contained 250 μCi of [^{35}S]cysteine per ml (1200 Ci/mmol; Amersham) during the last 6 hr of this incubation. The cells were then washed twice with cold Hanks' balanced salt solution (GIBCO), harvested by gently scraping the flasks with a rubber policeman, and solubilized in a modified RIPA buffer [20 mM 3-(*N*-morpholino)propanesulfonic acid/150 mM NaCl/1 mM EDTA/1% Nonidet P-40/1% deoxycholate/0.1% SDS] containing phenylmethylsulfonyl fluoride (174 μg/ml; Sigma), leupeptin (0.5 μg/ml; Sigma), and aprotinin (2 μg/ml; Boehringer Mannheim). After one freeze–thaw step, lysates from untreated and IL-1-treated cells were precleared with nonimmune mouse IgG (Sigma) and protein G-Sepharose beads (Pharmacia) for 1 hr at 4°C. Centrifugation supernatants were divided into two aliquots and incubated at 4°C for 1 hr with either nonimmune mouse IgG or anti-bovine HBGF-2 monoclonal IgG (Upstate Biotechnology, Lake Placid, NY). The samples were incubated with protein G-Sepharose beads for 1 hr at 4°C. The beads were washed five times with modified RIPA buffer and once with modified RIPA buffer containing 2.5 M KCl. Immunocomplexes were resuspended in 0.125 M Tris·HCl (pH 6.8)/4% SDS/20% (vol/vol) glycerol/10% (vol/vol) 2-mercaptoethanol (Sigma), heated at 95°C for 5 min, and analyzed on a SDS/15% polyacrylamide gel. The gel was fixed in 40% (vol/vol) methanol/10% (vol/vol) acetic acid and treated with Enlightning (New England Nuclear) prior to autoradiography. Autoradiographic signals were quantitated by laser densitometry; the degree of HBGF-2 protein induction was calculated after normalization to the intensity of an ≈32-kDa protein found in all four immunoprecipitation samples.

RESULTS AND DISCUSSION

IL-1α, IL-1β, and TNF-α Increase HBGF-2 mRNA Levels in HSVSMCs. Previous studies have demonstrated that both IL-1 and TNF-α promote DNA synthesis (28–30) and induce IL-1β (31) and IL-6 (32) gene expression in human smooth muscle cells. To investigate whether cytokines could regulate HBGF-1 or HBGF-2 gene expression, HSVSMCs were rendered quiescent by serum deprivation and treated for 4 hr with recombinant IL-1α, IL-1β, IL-6, or TNF-α. RNA was prepared and HBGF mRNA levels were assayed by RNA gel blot hybridization analysis. IL-1β mRNA expression was also examined as a positive control for IL-1 and TNF-α activity, and in this and all other RNA analyses we controlled for RNA loading by rehybridization to a GAPDH probe. HBGF-1 transcripts of the appropriate size (17) were expressed at a similar level in both quiescent and cytokine-treated HSVSMCs (data not shown). In contrast, IL-1α, IL-1β, and TNF-α increased HBGF-2 mRNA levels; as estimated by densitometry, the amount of HBGF-2 mRNA was increased 4.5-fold by both IL-1 polypeptides and 1.3-fold by TNF-α (Fig. 1*A*). Our size estimates for the major HBGF-2 mRNAs in HSVSMCs (7.0 and 3.7 kb) agree with those reported for other cell types (13–15). Three smaller-sized transcripts can also be detected by using longer film exposures and have been reported by others (23, 24, 26). Since we have not observed differential regulation of these various transcripts, only the major mRNA species are shown in the figures. IL-1 and TNF-α also increased IL-1β mRNA levels, although detection of the TNF-α-induced IL-1β transcripts required longer film exposures than the one presented here. Our estimated sizes for the IL-1β and GAPDH mRNAs, 1.6 and 1.3 kb, respectively, also agree with those reported previously (31, 33). At the concentration used and at the time point examined, IL-6 did not increase HBGF-2 or IL-1β gene expression.

We focused our subsequent studies on the regulation of HBGF-2 gene expression by IL-1α, and for comparison we also analyzed IL-1α-induced IL-1β gene expression. IL-1 caused a concentration-dependent increase in HBGF-2 mRNA levels; induction was apparent at 0.25 ng/ml, the lowest concentration tested (data not shown). Since endotoxin treatment of HSVSMCs can induce IL-1β gene expression (34), we determined whether the increased HBGF-2 and IL-1β mRNA levels observed after IL-1 treatment were due to contaminating endotoxin in our recombinant IL-1 preparation. Two experimental approaches indicated that this was not the case. First, the lipopolysaccharide antagonist polymyxin B did not prevent IL-1-induced HBGF-2 or IL-1β

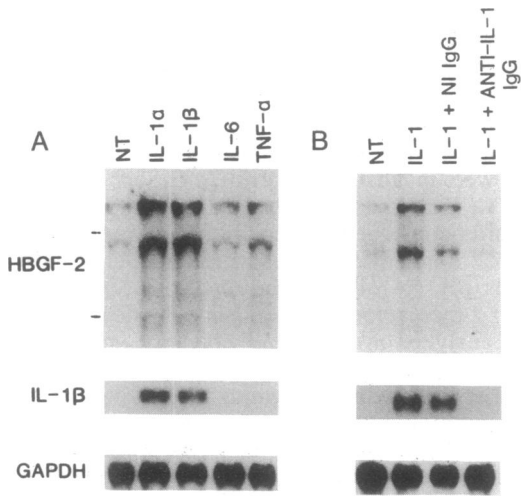

FIG. 1. Expression of HBGF-2 and IL-1β mRNA in cytokine-treated HSVSMCs. (*A*) Quiescent HSVSMCs either were left untreated (NT) or were treated for 4 hr with IL-1α (10 ng/ml), IL-1β (10 ng/ml), IL-6 (40 ng/ml), or TNF-α (20 ng/ml). RNA was prepared, and equivalent amounts of each sample were used for RNA gel blot analysis. In this and subsequent RNA gel blot figures, the cDNA probes used are indicated on the left, and the upper and lower tick marks on the left side of each HBGF-2 blot represent the positions of 28S and 18S rRNA, respectively. After obtaining optimal film exposures, the HBGF-2 blots were rehybridized to the GAPDH probe. (*B*) Quiescent HSVSMCs either were left untreated (NT) or were treated for 4 hr with IL-1α (1 ng/ml), IL-1α (1 ng/ml) preincubated with nonimmune (NI) rabbit IgG, or IL-1α (1 ng/ml) preincubated with rabbit anti-human IL-1α antibody. RNA was prepared, and equivalent amounts of each sample were used for RNA gel blot analysis.

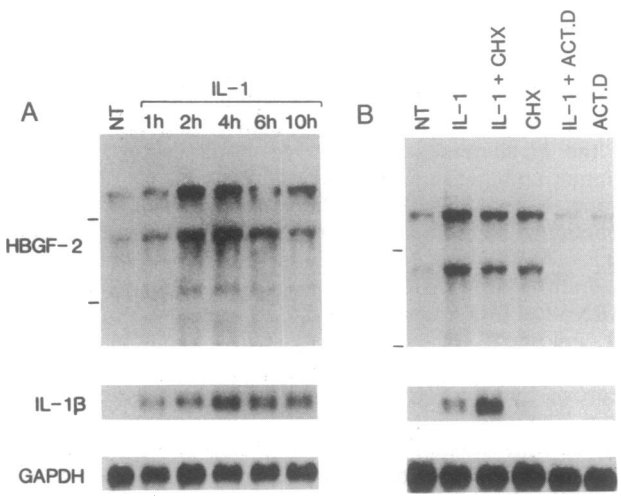

FIG. 2. Expression of HBGF-2 and IL-1β mRNA in IL-1-treated HSVSMCs. (*A*) Quiescent HSVSMCs either were left untreated (NT) or were treated with IL-1α (2 ng/ml) for the indicated times. RNA was prepared, and equivalent amounts of each sample were used for RNA gel blot analysis. (*B*) Quiescent HSVSMCs either were left untreated (NT) or were treated for 4 hr with IL-1α alone (10 ng/ml), IL-1α (10 ng/ml) and cycloheximide (CHX) (10 μg/ml), CHX alone, IL-1α (10 ng/ml) and actinomycin D (ACT.D) (2 μg/ml), or ACT.D alone. RNA was prepared, and equivalent amounts of each sample were used for RNA gel blot analysis.

mRNA expression (data not shown). Second, pretreatment of IL-1 with anti-IL-1 IgG, but not nonimmune IgG, blocked the ability of our IL-1 preparation to increase HBGF-2 and IL-1β mRNA levels (Fig. 1*B*).

IL-1 Induction of HBGF-2 mRNA Expression Does Not Occur if Transcription Is Inhibited and May Require *de Novo* Protein Synthesis. The kinetics of HBGF-2 mRNA induction were investigated by RNA gel blot analysis using RNA prepared from HSVSMCs treated with IL-1 for different periods of time. Increased HBGF-2 mRNA levels were evident 1 hr after IL-1 addition and peak expression was from 2 to 4 hr (Fig. 2*A*). At 10 hr poststimulation, the level of HBGF-2 mRNA was still above that observed in quiescent cells. Similar kinetics were observed for IL-1β mRNA expression and agree with the results reported by Warner *et al.* (31).

To determine whether *de novo* protein synthesis was required for HBGF-2 mRNA accumulation after IL-1 treatment, quiescent HSVSMCs were stimulated with IL-1 for 4 hr in the absence or presence of cycloheximide, an inhibitor of translation elongation. RNA was prepared and used in RNA gel blot hybridization experiments. HBGF-2 mRNA levels increased in response to the addition of IL-1 and cycloheximide together, although the level obtained was similar to that observed when cycloheximide alone was added (Fig. 2*B*). This result is in contrast to the effect of cycloheximide on IL-1-induced IL-1β gene expression. Cycloheximide alone had only a small effect on IL-1β mRNA levels, and treatment with both IL-1 and cycloheximide "superinduced" IL-1β mRNA levels. Similar cycloheximide effects on IL-1β mRNA induction have been observed in endotoxin-treated HSVSMCs (34). These results indicate that IL-1-induced HBGF-2 gene expression may require the synthesis of intermediary proteins, whereas IL-1-induced IL-1β gene expression does not.

The increase in HBGF-2 mRNA levels after IL-1 addition could be due to enhanced transcription and/or to increased transcript stability. It is probable that at least part of the response reflects transcriptional activation, since HBGF-2 mRNA levels do not increase if actinomycin D, an inhibitor of RNA synthesis, is included during IL-1 stimulation (Fig. 2*B*). As reported previously by Warner *et al.* (31), the induction of IL-1β mRNA expression by IL-1 also appears to require *de novo* RNA synthesis.

IL-1 Increases HBGF-2 Polypeptide Synthesis in HSVSMCs. We then determined whether IL-1 stimulation of HSVSMCs results in increased synthesis of HBGF-2 protein. Quiescent cells either were left untreated or were treated with IL-1 for 8.5 hr; [^{35}S]cysteine was included in the tissue culture medium during the last 6 hr of this incubation. The level of HBGF-2 synthesis in quiescent and IL-1-stimulated cells was assayed by immunoprecipitation with a HBGF-2-specific monoclonal antibody. Both quiescent and IL-1-stimulated HSVSMCs synthesized a polypeptide of ≈18 kDa that was immunoprecipitated specifically by this antibody (Fig. 3). The amount of immunoprecipitable protein was 2.5-fold higher in stimulated cells as estimated by densitometry. Therefore, it appears that the increased expression of HBGF-2 mRNA in IL-1-stimulated HSVSMCs is accompanied by increased HBGF-2 synthesis.

IL-1 Induction of HBGF-2 and IL-1β mRNA Expression Does Not Occur in the Presence of IFN-γ or Glucocorticoids. IL-1 can induce IL-1 gene expression in human blood monocytes (35, 36), endothelial cells (37), smooth muscle cells (31), and lung fibroblasts (38). This self-amplification system may lead to excessive IL-1 production and thus could be important in vascular pathology and in various chronic inflammatory diseases. The cytokine IFN-γ and the synthetic glucocorticoid dexamethasone can suppress IL-1-induced IL-1 production in monocytes (35, 36). IFN-γ and glucocorticoid hormones have numerous effects on vascular smooth muscle cell function. For example, they inhibit smooth muscle cell growth *in vitro* (39–42) and myointimal proliferation *in vivo* (40, 43). The antiproliferative effect of glucocorticoids may be important for their ability to inhibit atherosclerotic plaque

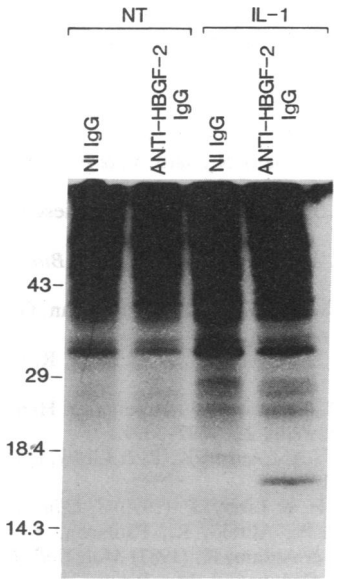

FIG. 3. HBGF-2 synthesis in untreated and in IL-1-treated HSVSMCs. An equivalent number of quiescent [no treatment (NT)] and IL-1α-treated (10 ng/ml) HSVSMCs were incubated in medium containing [^{35}S]cysteine and cell lysates were prepared. Immunoprecipitation using either nonimmune (NI) mouse IgG or mouse anti-bovine HBGF-2 antibody was performed as described. The immunoprecipitates were analyzed on a SDS/15% polyacrylamide gel and visualized by autoradiography. Molecular masses (in kDa) of ^{14}C-labeled protein size markers (BRL) are shown on the left.

formation in rabbits (44, 45). We investigated whether IFN-γ or glucocorticoids would inhibit IL-1-induced gene expression in HSVSMCs. Quiescent cells either were left untreated or were pretreated for 2 hr with IFN-γ, dexamethasone, or hydrocortisone. IL-1 was then added for 4 hr and the cells were collected. RNA was prepared and used for RNA gel blot hybridization analysis. IL-1 did not increase HBGF-2 or IL-1β mRNA levels when the cells were first treated with IFN-γ or glucocorticoids (Fig. 4).

IL-1 Induction of HBGF-2 mRNA Expression Also Occurs in Arterial Smooth Muscle Cells and Skin Fibroblasts, But Not in Endothelial Cells. The previous experiments were performed with venous smooth muscle cells. We determined whether arterial smooth muscle cells, which also proliferate in response to IL-1 (28, 29), expressed increased levels of HBGF-2 mRNA after IL-1 treatment. Quiescent HSVSMCs and aortic smooth muscle cells either were left untreated or were treated for 4 hr with an equivalent amount of IL-1. RNA was prepared and used for RNA gel blot analysis. Smooth muscle cells isolated by enzymatic dissociation from human aorta or from explants of saphenous vein showed a similar response to IL-1 (Fig. 5).

We then investigated whether the effect of IL-1 on HBGF-2 gene expression was cell type specific. For this analysis, we used two cell types that respond differently to IL-1 treatment. IL-1 is mitogenic for human fibroblasts (46, 47) but inhibits endothelial cell proliferation (47, 48). Quiescent human skin fibroblasts and umbilical vein endothelial cells either were left untreated or were treated for 4 hr with an equivalent amount of IL-1. RNA was prepared and used for RNA gel blot analysis. IL-1 was able to induce both HBGF-2 and IL-1β gene expression in human skin fibroblasts (Fig. 5). In contrast, IL-1 treatment of human umbilical vein endothelial cells did not increase HBGF-2 mRNA levels, although IL-1β gene expression was induced. A similar result was obtained by using human aortic endothelial cells (data not shown). In contrast to the other cell types tested, we could detect IL-1β mRNA in untreated endothelial cells. Also, the largest HBGF-2 transcript expressed in human umbilical vein endothelial cells was ≈6.4 kb, smaller than the 7.0-kb mRNA detected in the other cell types.

In conclusion, the results described above demonstrate that IL-1 can regulate HBGF-2 gene expression in smooth muscle cells and fibroblasts cultured *in vitro*. HBGF-2 is a potent mitogen for many cell types and an angiogenic factor. Thus, increased production of HBGF-2 in response to IL-1 *in vivo* may play a role in the pathogenesis of numerous diseases, including Kaposi's sarcoma, rheumatoid arthritis, and atherosclerosis. In regard to vascular pathology, leukocyte migration into the vessel wall occurs after vascular injury and during early stages of atherogenesis (reviewed in refs. 49 and 50). Indeed, a large proportion of the cells in human advanced atherosclerotic plaques are monocyte-derived macrophages and T lymphocytes (51, 52). IL-1 released from these cell types could induce additional IL-1 production by vascular endothelial (37) and smooth muscle (31) cells. Since the smooth muscle cells within atherosclerotic lesions express class II major histocompatibility antigens (53), it is likely that they interact with IFN-γ secreted by activated T lymphocytes. One would then predict that the level of IL-1 and HBGF-2 production by smooth muscle cells would depend on the relative amounts of IL-1 and IFN-γ within the vascular

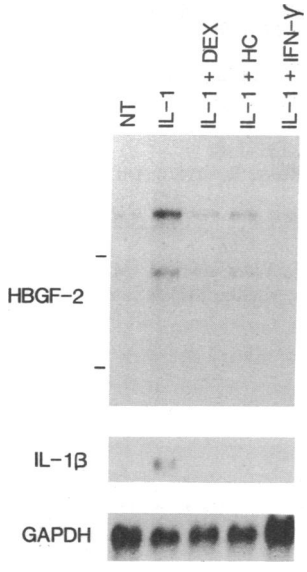

FIG. 4. Effect of glucocorticoids and IFN-γ on IL-1-induced HBGF-2 and IL-1β mRNA expression. Quiescent HSVSMCs either were left untreated (NT) or were treated for 2 hr with 2 μM dexamethasone (DEX), 200 μM hydrocortisone (HC), or IFN-γ (50 ng/ml). IL-1α (10 ng/ml) was then added to untreated or treated cultures for 4 hr. RNA was prepared, and equivalent amounts of each sample were used for RNA gel blot analysis.

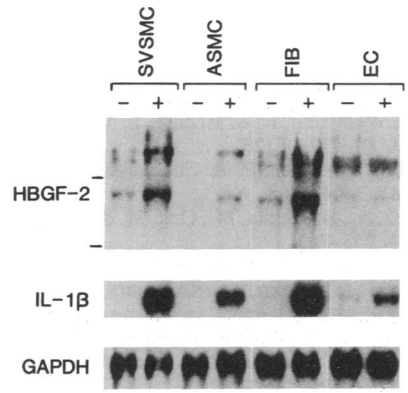

FIG. 5. Expression of HBGF-2 and IL-1β mRNA in IL-1-treated smooth muscle cells, fibroblasts, and endothelial cells. Quiescent human saphenous vein smooth muscle cells (SVSMC), aortic smooth muscle cells (ASMC), skin fibroblasts (FIB), or umbilical vein endothelial cells (EC) either were left untreated (lanes −) or were treated (lanes +) for 4 hr with IL-1α (10 ng/ml). RNA was prepared, and equivalent amounts of each sample were used for RNA gel blot analysis.

wall. It is of interest to note that Ross *et al.* (54) have shown recently that in comparison to normal aortas from control animals, atherosclerotic lesions of hypercholesterolemic animals contain high levels of IL-1β mRNA. Therefore, it is possible that HBGF-2 mRNA levels are also elevated in atherosclerotic plaques.

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