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Up-Regulation of Hepatocyte Growth Factor Gene Expression by Interleukin-1 in Human Skin Fibroblasts

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SUMMARY: Hepatocyte growth factor (HGF) functions as a hepatotrophic and renotrophic factor for regeneration of the liver and kidney. When 1 ng/ml of interleukin-1α (IL-1α) or interleukin-1β (IL-1β) was added to cultures of human skin fibroblasts, the production of HGF was 5-6 fold higher than levels in the controls. HGF mRNA level in the cells was increased to 4-fold higher levels at 6 h after exposure to IL-1α. Tumor necrosis factor-α and interferon-γ but no other cytokine tested had slightly stimulatory effects on HGF production. The tumor promoter, tetradecanoylphorbol 13-acetate (TPA) markedly enhanced the stimulatory effect of IL-1α and IL-1β on the production of HGF. The stimulatory effect of both IL-1α and IL-1β and the synergistical stimulation with TPA were completely abrogated by 10 ng/ml TGF-β1 or 1 μM dexamethasone. These results suggest that IL-1α and IL-1β are positive regulators for expression of the HGF gene and are likely have a role in regeneration of tissues following the occurrence of inflammatory diseases.

Hepatocyte growth factor (HGF), first found in the serum of partially hepatectomized rats was purified from rat platelets as a mitogen for mature hepatocytes in primary culture (1-3, and reviewed in Ref. 4, 5). HGF was also purified from human plasma (6, 7), CCl_4-administrated rat livers (8), and the conditioned medium of human lung fibroblasts (9). HGF is a heterodimer composed of the 69 kDa α-subunit and the 34 kDa β-subunit. Both human and rat HGF have been molecularly cloned and the sequence revealed four kringle domains in the α-chain and serine protease-like domain in the β-chain (10-13). High affinity cellular receptor for HGF with a Kd value of 20-30 pM has been

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detected in a wide variety of cells and tissues (14-16) and the HGF receptor has been identified as the c-*met* proto-oncogene product (17-19).

With the onset of various liver insults, there is a marked and rapid increase in HGF mRNA and HGF protein levels in the liver or plasma (6, 7, 20-22, and reviewed in Ref. 4, 5). We recently obtained evidence that intravenously injected recombinant HGF markedly enhanced liver regeneration in mice (23). On the other hand, a marked increase of HGF mRNA was also noted in the kidney following renal injuries (24). These results indicate that HGF acts both as a hepatotrophic factor and renotrophic factor in regeneration of the liver and kidney. Since HGF targets a wide variety of cells (4, 5, 9), it may possibly be involved in regeneration of various organs and tissues as a "trophic factor".

The "trophic" role of HGF in tissue regeneration appears to based on unique biological activities required to construct normal tissues. HGF acts as a mitogen for a variety of epithelial and endothelial cells (4, 5, 9, 25) and as a "scatter factor" which promotes the dissociation of epithelial and vascular endothelial cells *in vitro* (26, 27, and reviewed in Ref. 28). Moreover, HGF induces epithelial tubule formation *in vitro*, acting as an epithelial morphogen (29, 30). Because HGF is predominantly synthesized by stromal cells (4, 5, 31), this factor is probably the long-sought humoral mediator of epithelial-mesenchymal interactions (30, 32).

Based on all these findings, we addressed mechanisms regulating HGF gene expression and HGF production. We report here that interleukin-1α (IL-1α) and -1β (IL-1β) stimulate HGF synthesis in human skin fibroblasts, by enhancing expression of the HGF gene.

MATERIALS AND METHODS

Materials: Human recombinant HGF was purified from the conditioned medium of CHO cells transfected with expression vector containing human HGF cDNA (10, 13). Transforming growth factor-β1 (TGF-β1) was purified from human platelets as described elsewhere (33). Bovine acidic fibroblast growth factor (aFGF) and bovine basic fibroblast growth factor (bFGF) were kind gifts from Toyobo Co. (Osaka). Human recombinant interleukin-6 (IL-6) and human recombinant transforming growth factor-α (TGF-α) were kind gifts from Dr. T. Kishimoto (Osaka University) and Genentech Co. (South San Francisco), respectively. Human recombinant IL-1α, human recombinant IL-1β, human recombinant interleukin-2 (IL-2), human recombinant platelet-derived growth factor (PDGF), human recombinant tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and human recombinant nerve growth factor (NGF) were purchased from Genzyme Co. (Boston, MA). Tetradecanoylphorbol 13-acetate (TPA) and dexamethasone were purchased from Sigma (St. Louis, MO).

Cell culture: Normal human skin obtained during plastic surgery was cut into 0.5 mm pieces, plated onto culture dishes (Corning) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum (FCS). During 10 days of culture, the fibroblasts proliferated outward from the dermal tissue and the cells were passaged by conventional trypsinization and

further cultured in DMEM supplemented with 10% FCS. Seventh to tenth passaged cells were used for this study.

Measurement of HGF in culture medium: Human skin fibroblasts were plated on a 24-well plate (Coster) at a density of 5 x 10^4 cells/cm^2, and cultured for 24 h. After replacing the medium with fresh DMEM supplemented with 1% FCS, cytokines were added and the cells were cultured for 24 h. The concentration of HGF secreted into the culture medium was determined by enzyme-linked immunosorbent assay (ELISA), using rabbit anti-human polyclonal antibodies, as described elsewhere (34).

To determine cellular protein, the remaining cells were solubilized with 1 M NaOH and cellular protein was quantitated using a micro BCA protein assay kit (Pierce Chemical).

Northern blot hybridization: RNA was purified from cultured cells using the acid guanidium-thiocyanate-chloroform method (35). Twenty μg of total RNA were electrophoresed in a 1% agarose, 0.7% formaldehyde gel and transferred to a Hybond-N nylon membrane. The BamHI-SalI fragment of clone pBS-7 which includes the full length open reading frame cDNA for human HGF (10, 13), was labeled with [α-^{32}P]dCTP, using the multiprime labeling system, according to the manufacturer's instruction. The membrane was hybridized with radiolabeled cDNA at 42°C for 24 h in solution composed of 50% (v/v) formamide, 5 x SSPE (1 x SSPE consists of 0.15 M NaCl, 10 mM sodium phosphate buffer (pH 7.4), and 1 mM EDTA), 4 x Denhardt's, 0.55% SDS, and 150 μg/ml salmon sperm DNA. The filter was washed with 0.2 x SSPE containing 0.1% SDS for 15 min at 65°C, then was dried and autoradiographed. Densitometric analysis was carried out using a Bio-Image Analyzer.

RESULTS

We first examined effects of various cytokines on the production of HGF, by human skin fibroblasts (Table 1). Among the various cytokines tested, we found that IL-1α and IL-1β markedly stimulated the amount of HGF secreted into the medium of human skin fibroblasts. However, IL-2, IL-6, TGF-α, PDGF, aFGF, bFGF, and NGF had no effect on HGF production, while TNF-α and IFN-γ slightly stimulated HGF production but with a much lesser potential than seen with IL-1α and IL-1β. On the other hand, 10 ng/ml TGF-β1 and 1 μM dexamethasone suppressed HGF production to 20-30% and to 40-50% of control cultures, respectively. In addition to IL-1α and IL-1β, 10 nM TPA also stimulated HGF production with the same potential seen with IL-1α and IL-1β. Therefore, HGF production may be enhanced by activation of protein kinase-C.

Fig. 1 shows the dose-response curve of HGF production in the presence of IL-1α and IL-1β. IL-1 α and IL-1β stimulated HGF production, with a similar dose-dependency. The stimulatory effect was as low as 0.01 ng/ml and maximal stimulation by 5-6 fold occurred with 0.3-1.0 ng/ml.

Time course of the increase in HGF production after the addition of IL-1α and IL-1β is shown in Fig. 2. Stimulation of HGF production was not evident within 6 h after the addition of IL-1α or IL-1β. A significant stimulation of HGF production was seen at 9 h and marked stimulation of HGF production continued for up to 24 h, with less stimulation during the following 24 h.

Table 1. Effects of various cytokines, dexamethasone and TPA on HGF production in human skin fibroblasts

Samples	Concentration	HGF production* (ng/mg protein x 10^2)
None		7.0 ± 2.1
IL-1α	10 ng/ml	38.5 ± 4.1
IL-1β	10 ng/ml	44.3 ± 5.3
IL-2	10 ng/ml	6.5 ± 1.6
IL-6	10 ng/ml	7.4 ± 2.5
TGF-α	10 ng/ml	7.0 ± 1.0
TGF-β1	10 ng/ml	1.4 ± 0.1
PDGF	10 ng/ml	7.5 ± 2.5
TNF-α	10 ng/ml	12.3 ± 1.9
IFN-γ	10 ng/ml	10.0 ± 2.0
aFGF	10 ng/ml	7.1 ± 0.9
bFGF	10 ng/ml	6.8 ± 2.0
NGF	10 ng/ml	6.9 ± 1.0
Dex**	1 μM	3.2 ± 0.3
TPA	1 nM	7.6 ± 1.5
TPA	10 nM	36.8 ± 4.1

*Each value represents the mean (± SD) of triplicate measurements.
**Dexamethasone.

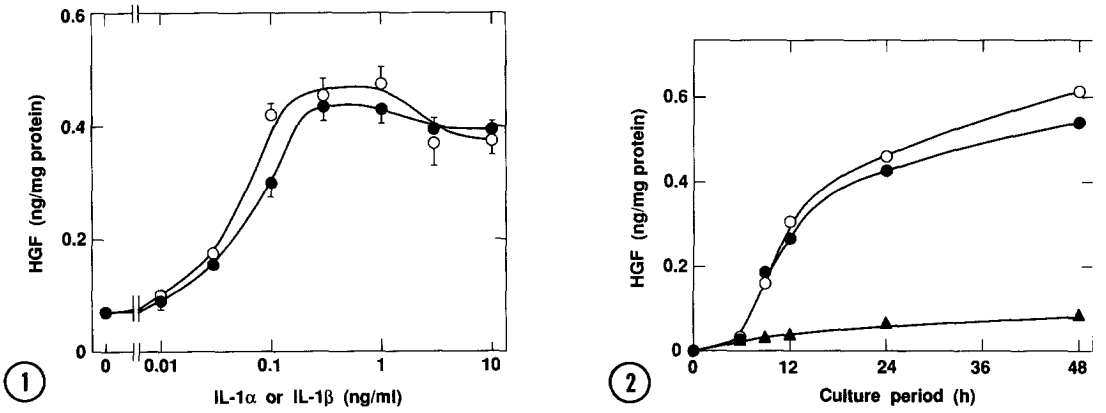

Fig. 1. Dose-responsive stimulation of HGF production in human skin fibroblasts by IL-1α and IL-1β. Human skin fibroblasts were plated on a 24-well plate at a density of 5 x 10^4 cells/cm^2 and cultured for 24 h. After the medium was changed to fresh medium, various concentrations of IL-1α (●) and IL-1β (○) were added and the cells were cultured for 24 h. The concentration of HGF in the medium was measured by ELISA. Each value represents the mean (± SD) of triplicate measurements.

Fig. 2. Time course of the increase of HGF production in human skin fibroblasts after addition of IL-1α and IL-1β. Human skin fibroblasts were plated on a 24-well plate at a density of 5 x 10^4 cells/cm^2 and cultured for 24 h. After the medium was changed to fresh medium, the cells were cultured in the absence (▲) or presence of 1 ng/ml IL-1α (●) or 1 ng/ml IL-1β (○) for the indicated period. The concentration of HGF in the medium was measured by ELISA. Each value represents the mean of triplicate measurements.

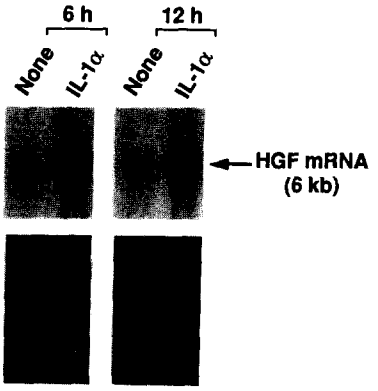

Fig. 3. Northern blot analysis of HGF mRNA in human skin fibroblasts. Human skin fibroblasts were cultured in the absence or presence of 1 ng/ml IL-1α for 6 h and 12 h. RNA was purified from the cells and 20 μg RNA per lane was electrophoresed in a 1.0% agarose/0.7% formaldehyde gel. RNA was transferred to a Hybond-N membrane and hybridized with [α-^{32}P]-labeled cDNA encoding full-length open reading frame of human HGF (10, 13). The membrane was washed (see Materials and Methods) then autoradiographed on X-ray film. Ribosomal RNAs stained with ethidium bromide are shown in the lower photograph, to indicate the amount of RNAs loaded onto the gel.

To determine whether the stimulation of HGF production by IL-1 α and IL-1β is due to enhancement of gene expression of HGF, we next examined HGF mRNA levels in skin fibroblasts following exposure to IL-1α (Fig. 3). The HGF mRNA increased to a 4-fold higher level during 6-12 h after the addition of IL-1 α. Therefore, the stimulation of HGF production by IL-1α and IL-1β is likely due to transcriptional activation of the HGF gene.

Fig. 4 shows effects of combinations of IL-1α, IL-1β and other factors on HGF production in skin fibroblasts. Although IL-1α or IL-1β alone increased HGF production to 5-6 fold higher levels than seen in the control cultures, HGF production was not further enhanced in the presence of both cytokines, thereby suggesting that IL-1α and IL-1β exert their effect through the same receptor. When IL-1α or IL-1β was concomitantly present with 10 ng/ml TGF-β1 or 1 μM dexamethasone, the stimulatory effect of IL-1α and IL-1β on HGF production was completely abrogated by TGF-β1 or dexamethasone.

Although 10 nM TPA alone stimulated HGF produciton with the same potential seen with IL-1α and IL-1β, it enhanced synergistically the stimulation of HGF production by IL-1α or IL-1β. HGF production in the presence of IL-1α plus TPA or IL-1β plus TPA was 20 fold higher than noted in the control cultures. It would thus appear that IL-1α and IL-1β stimulate HGF production through a signaling pathway distinct from that which activate protein kinase-C. Moreover, this synergistic stimulation of HGF production was almost completely abrogated by 10 ng/ml TGF-β1 or 1 μM dexamethasone.

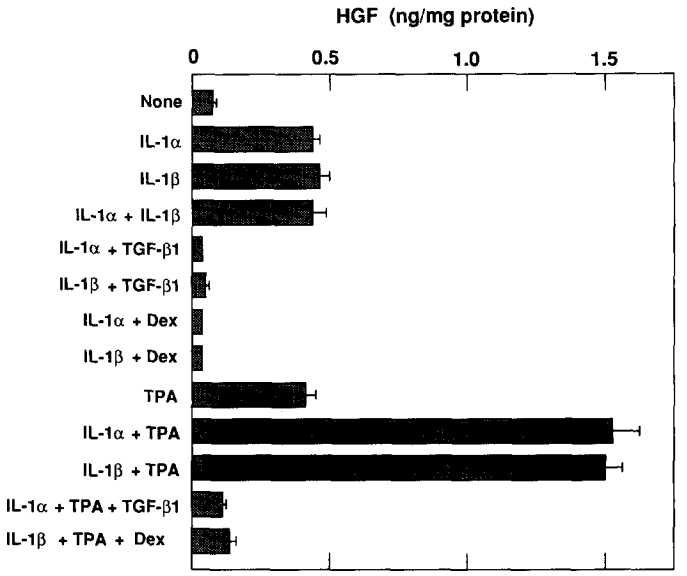

Fig. 4. Effects of combinations of IL-1α, IL-1β, and other factors on HGF production in human skin fibroblasts. Concentrations of compounds were as follows: IL-1α, 1.0 ng/ml; IL-1β, 1.0 ng/ml; TGF-β1, 10 ng/ml; dexamethasone (Dex), 1 μM; TPA, 10 nM. Human skin fibroblasts were plated on a 24-well plate at a density of 5 x 10^4 cells/cm^2 and cultured for 24 h. After the medium was changed to fresh medium, test samples were added and the cells were cultured for another 24 h. The concentration of HGF in the medium was measured by ELISA. Each value represents the mean (± SD) of triplicate measurements.

DISCUSSION

We obtained evidence that IL-1α and IL-1β stimulate HGF production in human skin fibroblasts and that this stimulatory effect is due to the enhancement of HGF gene expression. This is the first report of a positive regulation of HGF gene expression by IL-1α and IL-1β. Stimulatory effects of IL-1α and IL-1β on HGF production were also found in MRC-5 cells and HL-60 human promyelocytic leukemic cells, although the stimulation was not so marked as that in human skin fibroblasts (data not shown). In most cases, IL-1α and IL-1β possess the same biological activities and they share the same cell surface receptor (36), even though the homology in amino acid sequence between them is only 26% (37). Since IL-1α and IL-1β are indistinguishable regarding the stimulation of HGF production in skin fibroblasts, and the simultaneous addition of both cytokines did not produce any further increase in HGF production, the enhancement of HGF production by these cytokines may also be exerted through the same receptor molecule.

IL-1 is a multifunctional factor produced by macrophages, monocytes, neutrophills, vascular endothelial cells, fibroblasts, keratinocytes, and Kupffer cells (reviewed in Ref. 38). Among various biological activities, IL-1 has an

important role as an inflammatory cytokine to mediate acute phase reactions, such as induction of acute phase protein synthesis and prostaglandin synthesis. Therefore, based on our present finding, we postulate that IL-1α and IL-1β may be involved in regeneration of tissues subjected to inflammatory reactions, through their potential to induce expression of the HGF gene, or HGF may have a yet to be discovered important biological activity in immunological and inflammatory reactions.

Following hepatic or renal injuries, HGF mRNA increases rapidly not only in the injured organ but also in the intact lung and spleen (39, 40). We recently reported that "injurin", an inducer of gene expression of HGF, rapidly increases in serum of rats with hepatic or renal injury (34). Injurin is an acid- and heat-stable protein with an apparent molecular mass of 10-20 kDa and it is thought to induce expression of HGF in the injured organ and in intact organs distant from the injured site. As IL-1α and IL-1β have injurin-like activity, the question raises as to whether injurin is identical with either IL-1α or IL-1β. Although the complete primary structure of injurin will need to be determined, these factors appear to be distinct in biological activities and chemical properties. We consider that injurin may have a predominant role in tissue regeneration of various tissues and in tissue homeostasis by regulating the expression of HGF.

We also have data that TGF-β1 and glucocorticoids strongly suppress the expression of HGF gene in MRC human embryonic lung fibroblasts (submitted for publication). Therefore, the inhibitory effects of TGF-β1 and dexamethasone on HGF production in skin fibroblasts may be the result of diminished expression of the HGF gene.

Expression of the HGF gene appears to positively regulated by injurin, IL-1α, and IL-1β and negatively regulated by TGF-β1 and dexamethasone. Since HGF acts as a mitogen, as a motogen (stimulation of cell motility) and a morphogen (induction of epithelial tubule formation), this factor is considered to have a critical role in tissue organization during embryogenesis, organogenesis, as well as organ regeneration. Knowledge of the regulatory mechanism of HGF will shed light on the molecular mechanisms involved in tissue organization and regeneration.

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