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Functional Effects of FGF-13 on Human Lung Fibroblasts, Dermal Microvascular Endothelial Cells, and Aortic Smooth Muscle Cells

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We studied the effects of FGF-13 and FGF-2 on human lung fibroblasts, dermal microvascular endothelial cells, and aortic smooth muscle cells. FGF-13 induced cell growth of lung fibroblasts and aortic smooth muscle cells but had no effect on dermal vascular endothelial cells. FGF-2 induced cell growth in all the three cell types. FGF-13 and FGF-2 had little effect on IL-6 production by lung fibroblasts and aortic smooth muscle cells and substantially enhanced that induced by IL-1α. In contrast, FGF-13 and FGF-2 had little effect on IL-6 production by dermal vascular endothelial cells, either alone or in synergy with IL-1α.

Basic fibroblast growth factor (FGF-2), the prototypic member of the family of fibroblast or heparin-binding growth factors, was first identified and characterized on the basis of its ability to stimulate cell growth of ovarian cells (1). More recently, additional structurally related growth factors, each encoded by a distinct gene, have been identified (2,3). Fibroblast growth factors are potent regulators of cell growth, differentiation and function of a wide variety of cells derived from the desoderm and neuroectoderm. These proteins play crucial roles in normal development, in maintenance of tissues and in wound healing and repair. They may also contribute to pathological conditions, including tumor growth, rheumatoid arthritis, arteriosclerosis and metastases.

The FGFs vary in size from 155 to 268 amino acids, and share 33-65% amino acid sequence identity. FGFs mediate cellular responses by binding and activating of specific cell surface tyrosine kinase receptors (4). FGF-8 was demonstrated to be selective for FGFR3-IIIc and FGFR4 in comparison to FGFR1 and FGFR2 (5-7). FGF-2, however, binds to FGFR1-4. We compared the effects of FGF-13, a FGF-8 homolog (8-10), and FGF-2 on cell growth and IL-6 production in human lung fibroblasts, aortic smooth muscle cells and dermal microvascular endothelial cells. We found that FGF-13 is as effective as FGF-2 in causing cell growth of lung fibroblasts but at a 10-100X higher concentration. FGF-13 and FGF-2 also enhanced IL-1α induction of IL-6 release from lung fibroblasts and aortic smooth muscle cells but, in contrast to FGF-2, FGF-13 did not have any effect on proliferation of dermal microvascular endothelial cells.

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MATERIALS AND METHODS

Materials. Bovine serum albumin and indomethacin were from Sigma (St. Louis, MO). FGF-2 was from R&D Systems (Minneapolis, MN). IL-1α was a gift from Roche (Basel, Switzerland). FGF-13 (HODAH63) protein, was produced in E. coli transformant as two bands in modest amount with IPTG induction as reported previously (8).

Cells. Human lung fibroblasts and aortic smooth muscle cells were obtained and maintained in growth media from Clonetics (San Diego, CA). Human dermal microvascular endothelial cells were obtained and maintained in growth media from Cell Applications (San Diego, CA).

Cell growth assay. Human lung fibroblasts, aortic smooth muscle cells and dermal microvascular endothelial cells were cultured at 3,000 cells/well in a 96-well plate for one day in growth medium. Cells were then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA basal medium, the cells were incubated with the test proteins for 3 days. AlamarBlue (Alamar Biosciences, Sacramento, CA) was added to each well to a final concentration of 10% to measure cell growth. After 4 hours of incubation, fluorescence intensity of each well was measured by reading in a CytoFluor fluorescence reader (PerSeptive Biosystems, Framingham, MA).

PGE_2 assay. Human lung fibroblasts were cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells were incubated with FGF-2 or FGF-13

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with or without IL-1α for 20 hours. The supernatants were collected and assayed for PGE_2 by EIA kit (Cayman, Ann Arbor, MI).

IL-6 assay. Human lung fibroblasts, aortic smooth muscle cells and dermal microvascular endothelial cells were cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA medium, the cells were incubated with FGF-2 or FGF-13 with or without IL-1α for 20 hours. The supernatants were collected and assayed for IL-6 using two match-paired anti-IL-6 antibodies (Endogen, Cambridge, MA).

RESULTS

Stimulation of human lung fibroblast cell growth by FGF-13. Human lung fibroblasts were cultured with FGF-2 or FGF-13 for 3 days in basal medium before the addition of alamarBlue to assess their effect on growth of the fibroblasts. FGF-2 enhanced cell growth of the lung fibroblasts at 1-100 ng/ml while FGF-13 stimulated at 1,000-2,500 ng/ml (Figure 1A). However, the maximal effect of FGF-13 was similar to FGF-2. FGF-13 did not have any stimulatory effect on the dermal microvascular endothelial cells while FGF-2 did (Figure 1B). Both FGF-13 and FGF-2 induced a slight cell growth of aortic smooth muscle cells (Figure 1C).

Synergistic effect of FGF-13 on IL-6 and PGE_2 release. FGF-2 and FGF-13 did not have any effect on PGE_2 and IL-6 release from lung fibroblasts. However, both FGF-2 and FGF-13 acted synergistically with IL-1α to release PGE_2 (Figure 2A) and IL-6 (Figure 2B) from the fibroblasts. FGF-13 at 2,500 ng/ml produced a similar effect as 100 ng/ml FGF-2. Indomethacin (100 ng/ml), an inhibitor of PGE_2 synthesis, inhibited PGE_2 release but not IL-6 release induced by IL-1α from the fibroblasts.

Since the effects of FGF-2 and FGF-13 on PGE_2 and IL-6 release from the fibroblasts were similar we subsequently measured only IL-6 release from the aortic smooth muscle cells and dermal vascular endothelial cells. Neither FGF-13 nor FGF-2 alone had any effect on IL-6 release from aortic smooth muscle cells or dermal vascular endothelial cells. While the stimulation of IL-6 release from dermal vascular endothelial cells by IL-1α was not affected by FGF-2 or FGF-13 (Figure 3) both FGF-2 and FGF-13 enhanced the release of IL-6 from the aortic smooth muscle cells induced by IL-1α (Figure 4).

Effect of FGF-13 and FGF-2 on cell growth in the presence of IL-1α. The enhancing effect of FGF-13 and FGF-2 on IL-1α induction of IL-6 release may be due to their effect on cell growth. Therefore, we tested their co-incubation with IL-1α on cell growth by fibroblasts. There was no additive effect of either FGF-2 or FGF-13 with IL-1α (Figure 5). In aortic smooth muscle cells, both FGF-13 and FGF-2 stimulation of cell growth in the presence or absence of IL-1α were similar to those observed in the fibroblasts (Figure 6).

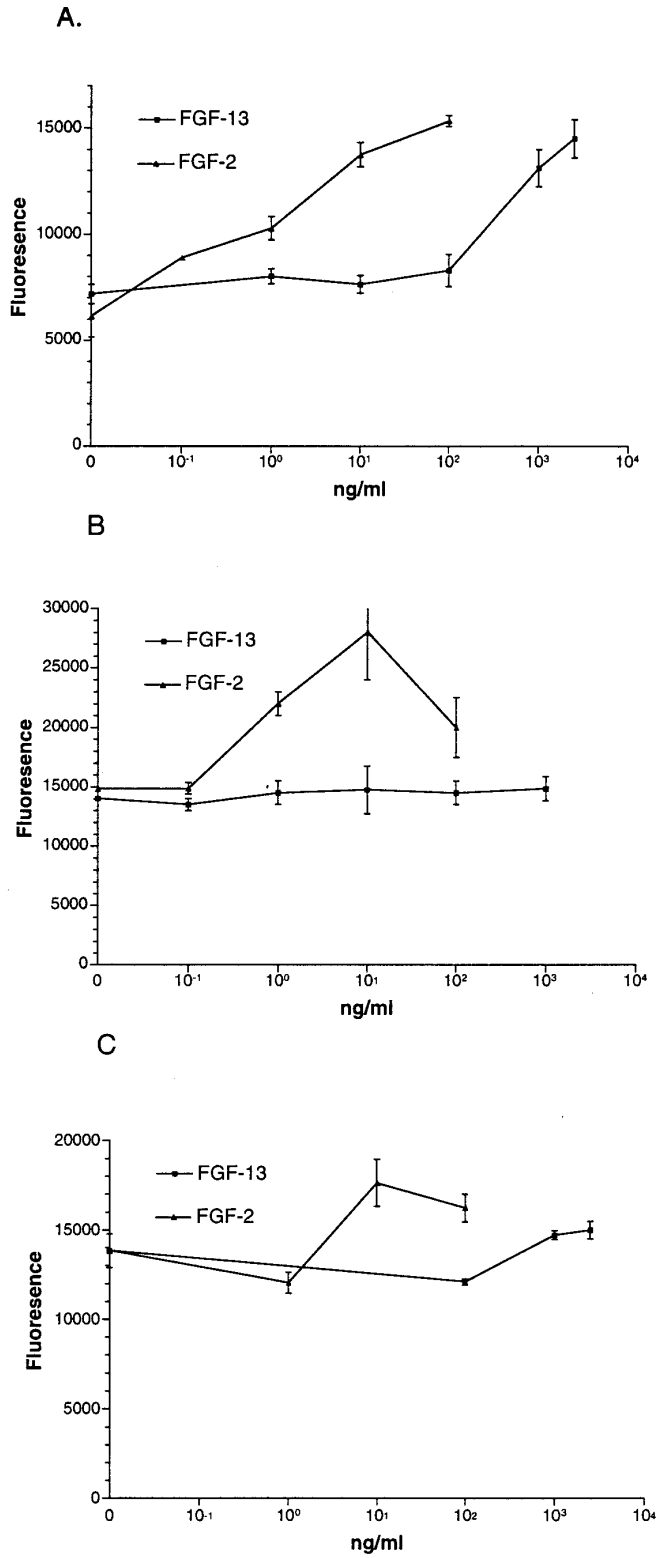

FIG. 1. Effect of FGF-13 and FGF-2 to induce cell growth of (A) human lung fibroblasts (B) human dermal microvascular endothelial cells, and (C) human aortic smooth muscle cells.

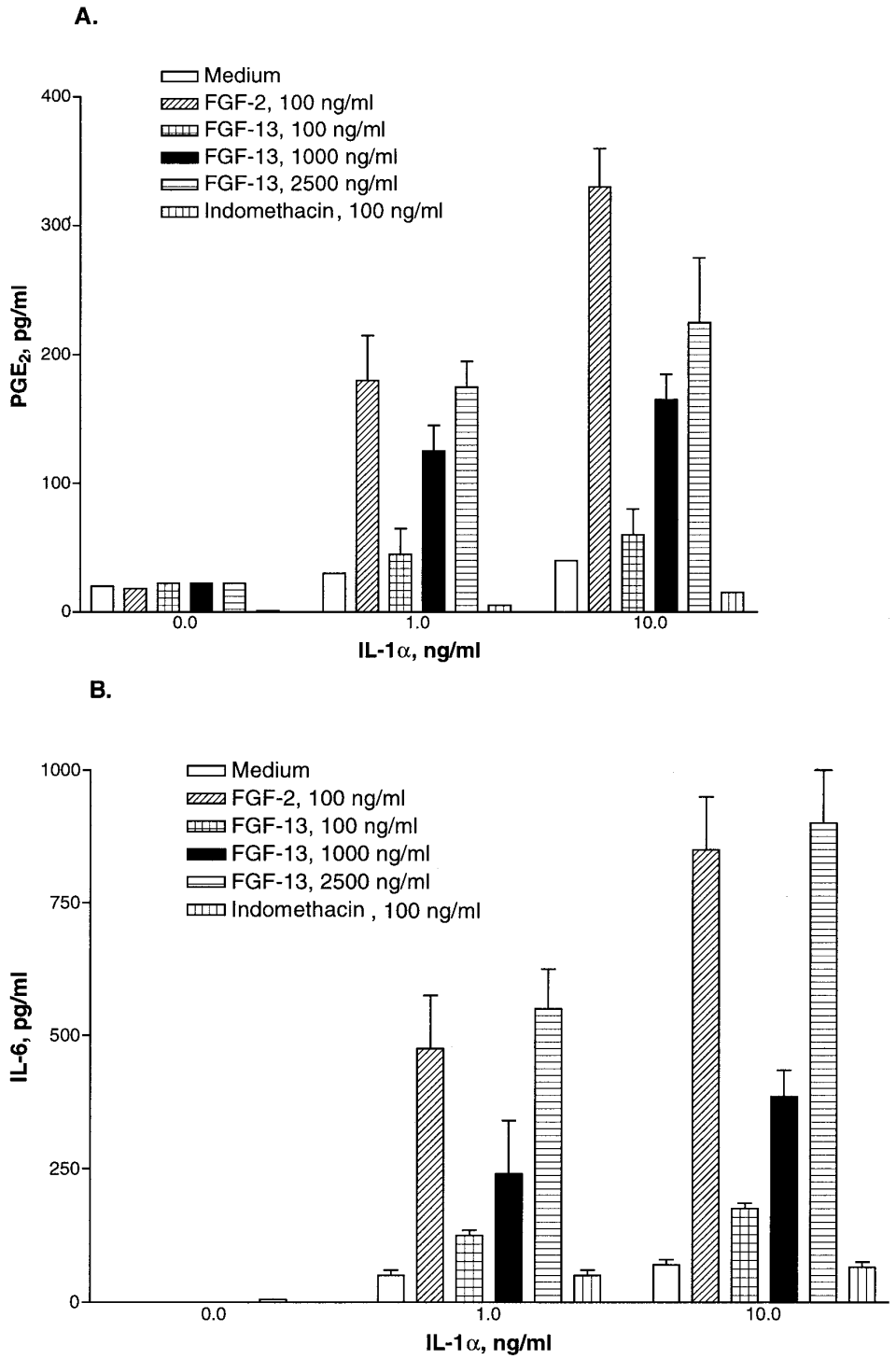

FIG. 2. (A) FGF-13 and FGF-2 act synergistically with IL-1α to induce PGE_2 release from the human lung fibroblasts. (B) FGF-13 and FGF-2 act synergistically with IL-1α to induce IL-6 release from the human lung fibroblasts.

In dermal vascular endothelial cells, IL-1α abrogated the stimulation of FGF-2, while FGF-13 had no effect on cell growth either alone or in combination with IL-1α (Figure 7).

DISCUSSION

FGF-13 acts similarly to FGF-2 in stimulating cell growth of human lung fibroblasts and aortic smooth

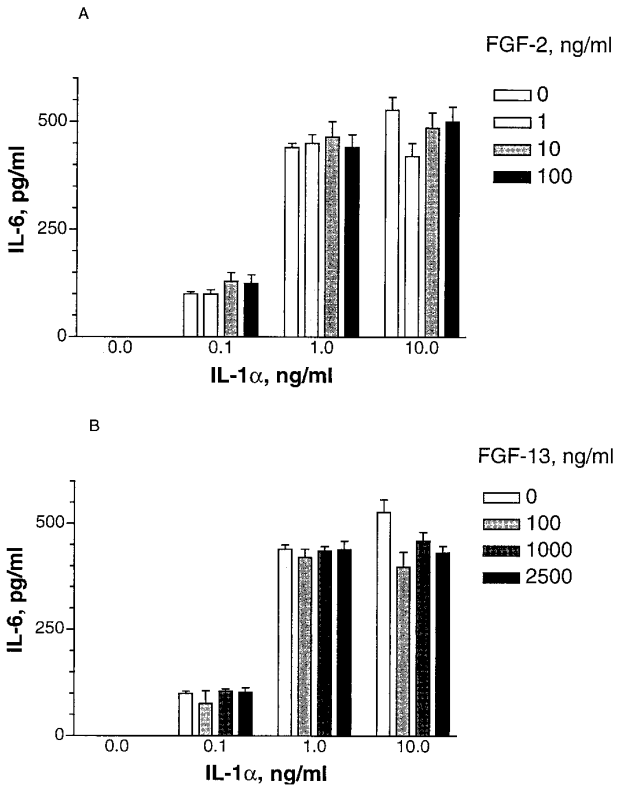

FIG. 3. FGF-13 and FGF-2 do not act synergistically with IL-1α to induce IL-6 release from the human dermal microvascular endothelial cells.

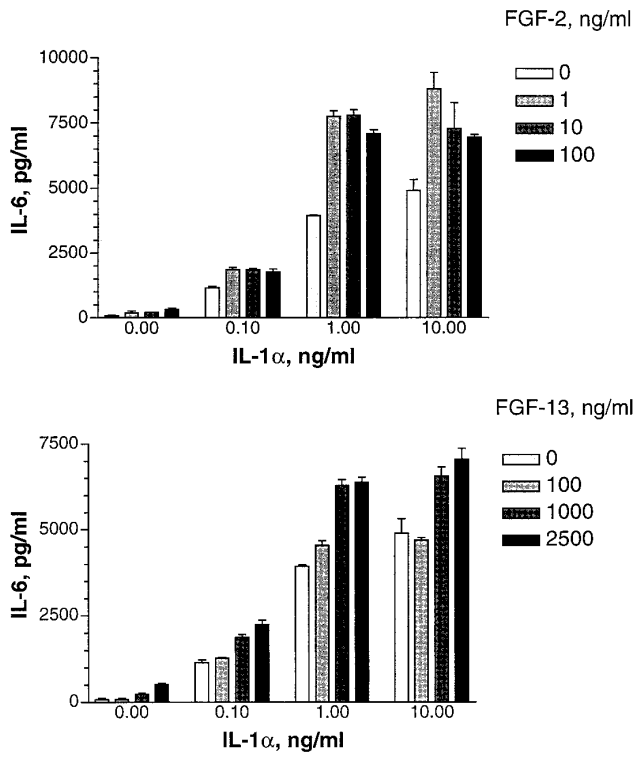

FIG. 4. FGF-13 and FGF-2 act synergistically with IL-1α to induce IL-6 release from the human aortic smooth muscle cells.

muscle cells. However, FGF-13 is 10-100 folds less potent than FGF-2. Both FGF-2 and FGF-13 had no effect on IL-6 or PGE_2 release from fibroblasts but acted synergistically with IL-1α. FGF-13 did not stimulate cell growth in dermal microvascular endothelial cells that were stimulated by FGF-2. FGFs bind and activate high-affinity receptor tyrosine kinases. The cloning of FGF receptors (FGFRs) has identified four distinct genes (5). FGFRs1-3 contain alternatively spliced Ig-like domains "b" and "c" that are expressed on the cell surface. FGFR4 is not spliced. FGF-2 binds mainly to FGFR1b, FGFR1c, FGFR2c, FGFR3c and FGFR4. FGF-8 binds mainly to FGFR3c and FGFR4 and slightly to FGFR2c. FGF-13 is a homolog of FGF-8. The difference of activity on the endothelial cells and fibroblasts may be due to the inability of FGF-13 to bind efficiently to FGFR1-2 and the lacking of FGFR3 and FGFR4 on the endothelial cells.

FGF-2 and FGF-13 did not stimulate fibroblasts to release PGE_2 or IL-6 but did enhance the IL-1α induction of PGE_2 and IL-6 release in 20 hours. A similar synergistic effect of FGF-2 and IL-1 on PGE_2 release from rheumatoid arthritis synovial cells was reported (11-12). The synergistic effect of FGF-2 and FGF-13 on IL-1α-induced IL-6 production was not due to an

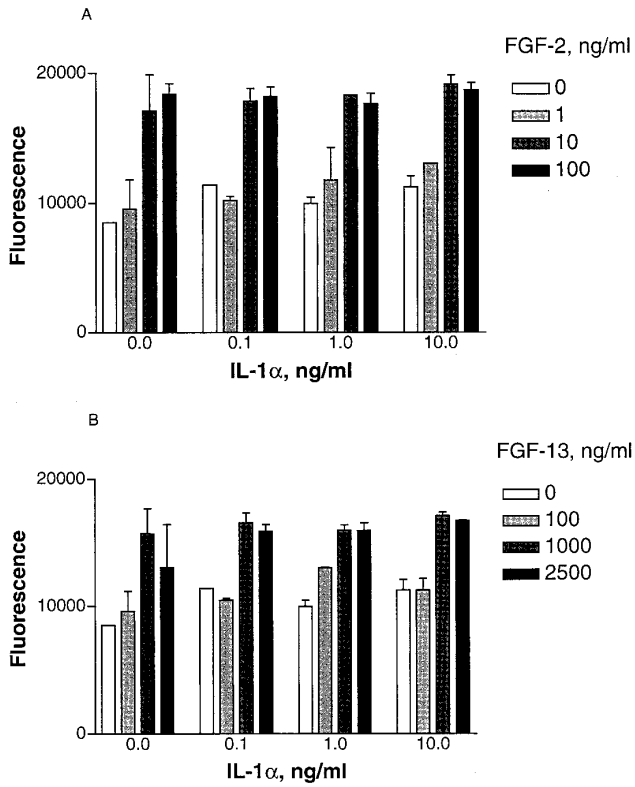

FIG. 5. FGF-13 and FGF-2 do not act synergistically with IL-1α to induce cell growth of the human lung fibroblasts.

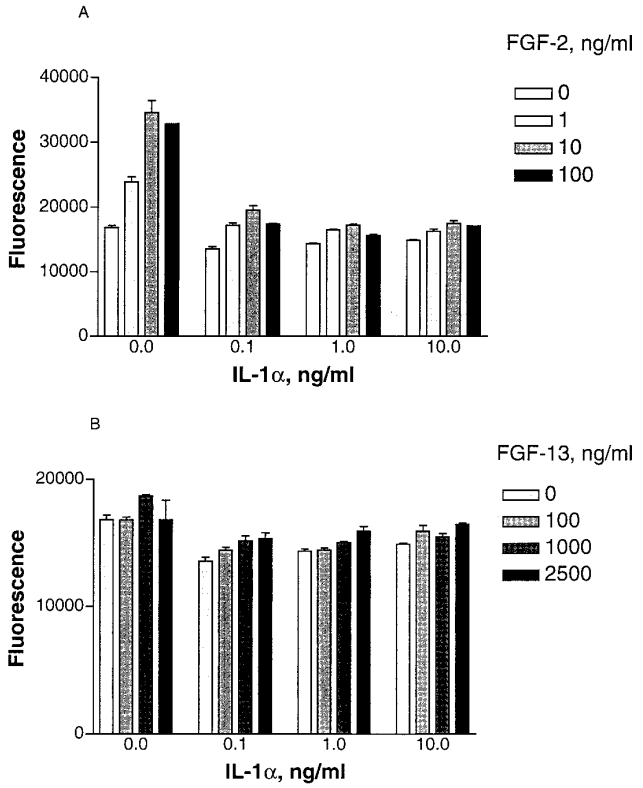

FIG. 6. IL-1α inhibits FGF-2 to induce cell growth of the human dermal microvascular endothelial cells. FGF-13 has no effect on cell growth.

increase in cell growth of the fibroblasts. FGF-2 and FGF-13 did not have any effect on the IL-1α induction of cell growth of the fibroblasts. Our data suggest that FGF-2 and FGF-13 may play a role in the regulation of IL-1 receptor expression on cells. Increased expression of IL-1 receptors could lead to increased synthesis of PGE_2 and IL-6. They may also act by activation of enzymes involving PGE_2 and IL-6 synthesis. IL-1α was shown to induce its own receptor on human fibroblasts by increasing endogenous levels of PGE_2 (11-12). Cells from inflamed gingiva contained an increased in receptors on their cell surface as compared to cells from healthy gingiva (13). Recently, the neuropeptide substance P was found to be directly regulating IL-1 receptor expression on bone marrow fibroblasts leading to their enhanced proliferation (14). Endothelin augmented a production of IL-6 and synergized with IL-1 to increase IL-6 secretion in HUVEC (15).

FGF-2 stimulated cell growth of dermal vascular endothelial cells. IL-1α abrogated this effect. However, the IL-1α induction of IL-6 was not affected by FGF-2. FGF-13 did not have any effect on cell growth or IL-6 secretion in dermal vascular endothelial cells. FGF-13 also had no effect on IL-1α induction of IL-6. These data suggest that endothelial cells contain a different set of FGFRs than fibroblasts and therefore, respond differently to various FGFs. It is also feasible that the level of high affinity receptors (FGFR3 and FGFR4) for FGF-13 may differ on the human lung fibroblasts, dermal microvascular endothelial cells and aortic smooth muscle cells. It was reported that rat cortical neuronal cells contain high affinity receptor for FGF-13 (8, 16) and respond better to FGF-13 than FGF-2 in various assays.

FGF-13, FGF-2 and IL-1α caused a slight cell growth of aortic smooth muscle cells. FGF-13 and FGF-2 induced a further cell growth as induced by IL-1α. FGF-13 and FGF-2 had little effect on IL-6 production alone but the co-incubation of IL-1α with FGF-2 or FGF-13 produced a synergistic release of IL-6 from aortic smooth muscle cells. The maximal effect of FGF-2 was at 1 ng/ml and 1000 ng/ml for FGF-13. The aortic smooth muscle cells may contain fewer receptors for FGF-2 and FGF-13 than the lung fibroblasts.

In summary, FGF-13 enhances cell growth of lung fibroblasts and to a lesser extent of aortic smooth muscle cells. It has no effect on cell growth of dermal vascular endothelial cells. This is a marked difference from FGF-2, which stimulates their cell growth. FGF-13 has no effect of IL-6 production from the three cell types

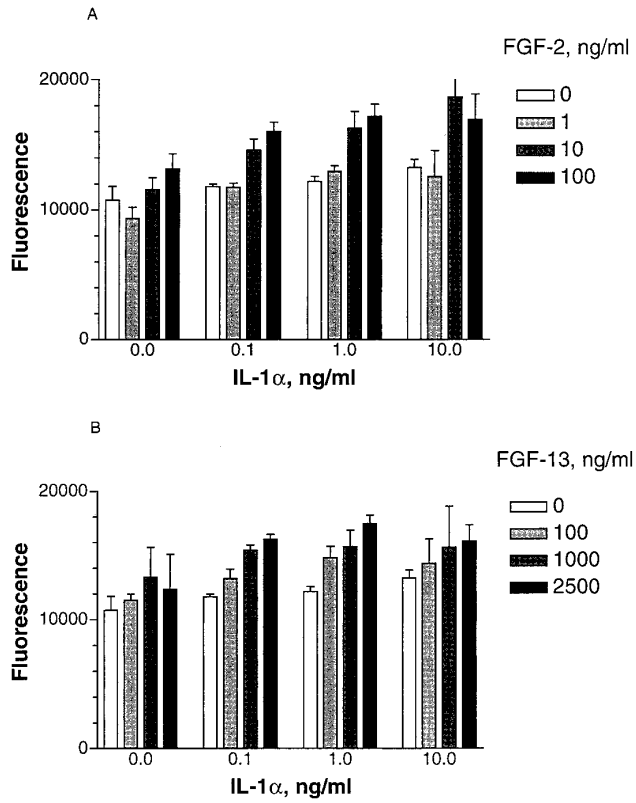

FIG. 7. FGF-13 and FGF-2 do not act synergistically with IL-1α to induce cell growth of the human aortic smooth muscle cells.

by itself but acts synergistically with IL-1α on the fibroblasts and aortic smooth muscle cells. FGF-13 has no effect on IL-6 production from the dermal vascular endothelial cells induced by IL-1α.

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