The effects of human recombinant interleukin (IL)-1β on elastin gene expression were studied in human skin fibroblast cultures by Northern hybridization and transient transfection experiments. Incubation of the cells with IL-1β elevated the elastin mRNA steady-state levels by ~3- to 4-fold. A similar increase was noted at the protein level, when estimated by indirect immunofluorescence of cultured cells. This effect was independent of the ongoing protein synthesis, as tested by incubation with cycloheximide. Transient transfections of the dermal fibroblasts with a human elastin promoter/chloramphenicol acetyltransferase (CAT) reporter gene construct suggested transcriptional regulation, since the CAT activity in cells incubated with IL-1β was similarly increased ~3-fold. Enhancement of the human elastin promoter activity by IL-1β was also noted in fibroblast cultures established from the skin and lungs of transgenic mice which had integrated the human promoter/CAT construct into their genome and express it in a tissue-specific manner. Furthermore, subcutaneous injection of IL-1β to the mice resulted in a ~4-fold elevation of the CAT activity in the skin after a 30-h incubation, as compared to the CAT activity in the skin of control animals. Collectively, these data indicate that IL-1β up-regulates elastin gene expression in fibroblast cultures as well as in the skin in vivo, and the activation occurs at the transcriptional level.

The major component of the elastic fibers, elastin, is initially synthesized as 70-kDa polypeptides which polymerize into an insoluble fiber network through the formation of characteristic cross-link compounds, desmosines (reviewed in Uitto et al. (1991)). The elastin polypeptides have been shown to be synthesized by a variety of cell types both in vivo and in vitro. In arterial blood vessels, the smooth muscle cells appear to be the major cell type responsible for elastin synthesis, but in other tissues, fibroblastic cells have been clearly shown to express the elastin gene. The 70-kDa polypeptide is encoded by a 3.5-kb mRNA, which is transcribed from a ~45-kb gene mapped to chromosome 7 in the human genome (Bashir et al., 1989; Fazio et al., 1991). The human elastin gene consists of 34 separate exons (Bashir et al., 1989), and there is considerable evidence for alternative splicing, leading to synthesis of different elastin isoforms (Indik et al., 1987; Fazio et al., 1988).

Elucidation of the 5′-flanking region of the human elastin gene has revealed the presence of several putative cis-regulatory elements (Fazio et al., 1990; Kähari et al., 1990). Furthermore, a variety of cytokines, growth factors, and hormones have been shown to modulate elastin gene expression, including transforming growth factor-β (Liu et al., 1988; Kähari et al., 1992a), tumor necrosis factor-α (Kähari et al., 1992b), insulin-like growth factor-1 (Foster et al., 1987; Badesc et al., 1989), glucocorticoids (Mecham et al., 1984), cyclic AMP (Rosenbloom et al., 1991), and vitamin D3 (Pierce et al., 1992). Recently, recombinant interleukin-1β (IL-1β) has been shown to inhibit elastin formation in a subset of fibroblasts cultured from neonatal rat lung (Berk et al., 1991). In general, the mechanisms of the modulation of elastin gene expression by these factors are not well delineated.

In this study, we have examined the effects of human recombinant IL-1β on elastin gene expression in human skin fibroblasts in culture, as well as in mouse skin and lung fibroblast cultures established from transgenic mice expressing the human elastin promoter linked to the CAT reporter gene. Furthermore, the effect of IL-1 was tested in vivo by subcutaneous injection of these transgenic mice. The results indicate consistent up-regulation of elastin gene expression at the transcriptional level.

**MATERIALS AND METHODS**

**Cell Cultures**—Normal human dermal fibroblasts, cultured by explanting tissue specimens obtained during surgical procedures, were utilized in passages 3-8. Transgenic fibroblasts were obtained by explanting skin or lung fragments from transgenic mice which have
Interleukin-1β Up-regulates Elastin Gene Expression

5.2 kb of 5′-flanking region of the human elastin gene, linked to the chloramphenicol acetyltransferase (CAT) reporter gene. The site of integration of the human elastin promoter/CAT construct within the mouse genome has not been determined, but the inheritance of the human construct clearly follows an autosomal dominant pattern in the mouse line. This construct contains elastin gene promoter sequences sufficient to confer its tissue-specific expression, as determined by the assay of CAT activity in various organs (Hsu-Wong et al., 1992). The cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mM glutamine, and antibiotics. Human recombinant IL-1β was purchased from Boehringer Mannheim and was added to the cell cultures in varying concentrations (see “Results”).

Northern Analyses—Adult skin fibroblasts in confluent monolayer cultures were incubated with or without IL-1β, and total RNA was isolated as previously described (Chirgwin et al., 1979). RNA was analyzed by Northern hybridizations with 32P-labeled cDNA probes (Sambrook et al., 1989) for human elastin (Fazio et al., 1988) and pro-collagen sequences (Chu et al., 1982), respectively. A glyceraldehyde-3-phosphate dehydrogenase cDNA was used in control hybridizations to normalize for the differences in the loading and transfer of RNA (Fort et al., 1985). The [32P]cDNA-mRNA hybrids were visualized by autoradiography, and the steady-state levels of mRNA were quantitated by scanning densitometry using a He-Ne laser scanner at 633 nm (LKBProduktor, Bromma, Sweden).

Indirect Immunofluorescence—For immunostaining, fibroblasts were cultured in glass chamber slides and fixed in cold (−20°C) ethanol. The slides were rinsed with Tris-buffered saline (pH 7.6), and blocked with 1% bovine serum albumin. The samples were exposed to a mouse monoclonal anti-human elastin antibody (IgG, Sigma) overnight at 4°C. The slides were then washed in Tris-buffered saline for 60 min and incubated with a tetramethylrhodamine isothiocyanate-conjugated secondary anti-mouse IgG antibody (Miles Laboratories). After a 60-min incubation at room temperature, the sections were washed with Tris-buffered saline for 60 min, rinsed with distilled water, mounted, and examined with a fluorescent microscope.

Treatment of Transgenic Mice—To test the effects of IL-1 on the elastin promoter activity in vivo, 100 units of recombinant human IL-1β in 200 μl of 0.15 M NaCl was injected subcutaneously into 5-day-old transgenic mice which we have recently developed and express the human elastin promoter linked to the CAT gene (see above). The control animals were injected with 200 μl of 0.15 M NaCl alone. After 30 h, the animals were sacrificed and a −1-cm2 area of the skin covering the site of the subcutaneous injection was removed. In addition, several internal organs, including the lungs, kidneys, and the heart, were dissected. The isolated tissues were homogenized with a Polytron tissue grinder, in combination with freeze-thawing of the homogenate three times. The homogenates were centrifuged at 10,000 × g for 15 min and aliquots of the supernatants were assayed for CAT activity, as described above.

Transient Transfections of Cultured Cells—Human neonatal foreskin fibroblasts in late logarithmic growth phase were transfected with 20 μg of plasmid DNA, pEP1/CAT, which contains 5.2 kb of 5′-flanking DNA of the human elastin gene cione into the promoterless plasmid pBSOCAT (Fazio et al., 1990; Kahari et al., 1990). This plasmid was generated by subcloning a 1.6-kb HindIII-BamHI fragment of pSV2CAT, containing the entire CAT gene, small t intron, and the polyadenylation signal, into the polylinker of the plasmid Bluescript pBSKS. The cells were co-transfected with a Rous sarcoma virus promoter/β-galactosidase construct to allow determination of the transfection efficiency (Sambrook et al., 1989). The specificity of the IL-1β effect on the elastin promoter was assayed by transfecting parallel cultures with pBSOCAT. The transfections were performed with the calcium-phosphate/DNA co-precipitation method (Graham and Van der Eb, 1973), followed by a 1-min (15%) glycerol shock. After the glycerol shock, the cells were placed in medium supplemented with 10% FCS. At the end of incubation, total RNA was extracted and analyzed by Northern hybridizations (15 μg/lane) with 32P-labeled cDNA probes for elastin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs. The faint upper band observed in the left panel reflects incomplete removal of the elastin probe prior to re-hybridization with the glyceraldehyde-3-phosphate dehydrogenase cDNA.

RESULTS AND DISCUSSION

Human skin fibroblasts in culture have been previously shown to express the elastin gene, as detected at the mRNA level (Giro et al., 1985; Olsen et al., 1988). The effects of recombinant IL-1β were first tested in fibroblast cultures established from adult skin. In control cultures, maintained in medium supplemented with 10% FCS, low, yet clearly detectable, levels of mRNA transcripts with the appropriate size of 3.5 kb were detected (Fig. 1). Addition of IL-1β (0.1–10 units/ml) resulted in a dose-dependent increase in the steady-state levels of elastin mRNA. Similar up-regulation of elastin mRNA levels could be observed in cultures maintained in medium containing only 1% FCS. Re-hybridization of the same filters with a glyceraldehyde-3-phosphate dehydrogenase cDNA allowed quantitative determination of the effect of IL-1β by scanning densitometry (Table I). Relatively little or no change was noted with 0.1 or 0.5 units/ml IL-1β, while incubation with 1, 5, or 10 units/ml resulted in 3–4-fold increases in the elastin mRNA levels (Table I). This stimulatory effect of IL-1 on elastin mRNA levels (up to ~4-fold) was consistently observed in 7 different fibroblast strains established from the skin of donors of varying ages (from 1 month to 73 years old). The mean stimulation among these cell strains was 3.2 ± 1.8 (mean ± S.D.). Thus, the elastin gene expression is clearly up-regulated by human recombinant IL-1β in cultured human skin fibroblasts, as determined at the mRNA level.

To demonstrate the up-regulatory effect of IL-1 on elastin gene expression at the protein level, fibroblasts were similarly incubated with IL-1β (1 and 10 units/ml) and accumulation of elastin was semiquantitatively assessed by indirect immunofluorescence. The results indicated significant increase in the elastin epitopes associated with the cells in cultures treated with IL-1 (Fig. 2). Thus, IL-1 increases elastin gene expression in cultured fibroblasts both at the mRNA and

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S. Hsu-Wong, manuscript in preparation.
Interleukin-1\(\beta\) Up-regulates Elastin Gene Expression

Human adult dermal fibroblasts were incubated with varying concentrations of human recombinant IL-1\(\beta\) (0.1–10 units/ml) for 24 h in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Total RNA was analyzed by Northern hybridization with cDNA probes for elastin and glyceraldehyde-3-phosphate dehydrogenase mRNAs. Quantitation of the specific transcripts was performed by scanning densitometry, and the elastin mRNA values were corrected for the glyceraldehyde-3-phosphate dehydrogenase mRNA levels in each RNA preparation. The values are expressed as relative densitometric units, and as a percent of control cultures incubated without IL-1\(\beta\).

<table>
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<th>IL-1(\beta) units/ml</th>
<th>Elastin mRNA levels</th>
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<tr>
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<td>densitometric units</td>
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<tr>
<td>0</td>
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<td>0.1</td>
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<tr>
<td>0.5</td>
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<td>1</td>
<td>2.02</td>
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<tr>
<td>5</td>
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<td>10</td>
<td>2.58</td>
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Previous studies have suggested that cytokines may have either direct effects on elastin gene expression, or synthesis of an intermediary protein is required for the effect (Berk et al., 1991; Kährä et al., 1992a). To distinguish between these two possibilities, further experiments were performed in which on-going protein synthesis was inhibited by the addition of cycloheximide prior to the addition of IL-1\(\beta\). As reported elsewhere, 10 \(\mu\)g/ml cycloheximide reduces fibroblast protein synthesis well over 90% under the conditions used (Otani et al., 1990). As expected from the experiments described above, incubation of adult skin fibroblasts with 1 unit/ml IL-1\(\beta\) increased the elastin mRNA levels by 3.4-fold, after correction for the glyceraldehyde-3-phosphate dehydrogenase mRNA abundance (Fig. 3). Addition of cycloheximide (10 \(\mu\)g/ml) to parallel cultures resulted in a ~2.0-fold increase in the elastin mRNA abundance, confirming previous observations (Berk et al., 1991). This effect may reflect stabilization of the elastin mRNA. However, the addition of cycloheximide to the culture medium 1 h prior to the addition of IL-1\(\beta\) had no effect on the up-regulation of elastin gene expression by this cytokine, and in fact, the elastin mRNA levels in the latter cultures were increased by ~4.0-fold, after correction for the glyceraldehyde-3-phosphate dehydrogenase mRNA in the same Northern blots, as compared with controls incubated without cycloheximide and IL-1\(\beta\) (Fig. 3). In contrast, rehybridization of the same filter with a human pro-\(\alpha\)(I) collagen cDNA indicated that the up-regulation of \(\alpha\)1 collagen gene expression elicited by IL-1\(\beta\) was abrogated by cycloheximide (Fig. 3). These observations suggest different mechanisms for the regulation of elastin and type I collagen gene expression by IL-1\(\beta\). Nevertheless, the results indicate that enhancement

![Fig. 2. Effects of IL-1\(\beta\) on elastin accumulation in fibroblasts, as assessed by indirect immunofluorescence. Preconfluent adult human dermal fibroblasts were incubated in Dulbecco’s modified Eagle’s medium supplemented with 10% FCS without or with IL-1\(\beta\) for 24 h. At the end of the incubation time, the cultures were fixed and elastin accumulation was estimated by indirect immunofluorescence with a monoclonal anti-human elastin antibody. A, control cells; B, cells incubated with 1 unit/ml IL-1\(\beta\); C, cells incubated with 10 units/ml IL-1\(\beta\).](image)

![Fig. 3. Effect of cycloheximide on IL-1\(\beta\)-induced up-regulation of elastin gene expression in human dermal fibroblasts. Confluent adult human dermal fibroblasts were incubated in Dulbecco’s modified Eagle’s medium supplemented with 1% FCS with or without cycloheximide (10 \(\mu\)g/ml) 1 h prior to the addition of IL-1\(\beta\) (1 unit/ml). Total RNA was extracted after a 24-h incubation and analyzed by Northern hybridization with \(^{32}\)P-labeled cDNAs for elastin (3.5 kb), pro-\(\alpha\)(I) collagen (5.8 and 4.8 kb), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1.3 kb) mRNAs. Lane 1, control; lane 2, IL-1\(\beta\); lane 3, cycloheximide; lane 4, IL-1\(\beta\) + cycloheximide. Note the strong specific 3.5-kb signal representing human elastin mRNA. The faint upper band represents nonspecific cross-hybridization to the 4.8-kb 18S ribosomal RNA.](image)
of the elastin gene expression by IL-1β in these cells is not dependent on active protein synthesis.

To further investigate the mechanisms by which elastin gene expression is enhanced in dermal fibroblasts, human neonatal skin fibroblasts were transfected with a human elastin promoter/CAT reporter gene construct, pEP1/CAT, which contains ~5.2 kb of 5'-flanking DNA of the human elastin gene (Kähari et al., 1990). Although the 5.2-kb 5'-flanking DNA of the elastin promoter region may not contain all regulatory elements of the elastin gene, our studies (Hsu-Wong et al., 1992) have established that this region is sufficient to confer tissue-specific expression of the gene, as compared with the endogenous expression of elastin in these animals. Transfection of the cells, followed by the addition of IL-1β (0.1–10 units/ml) 3 h later, resulted in a dose-dependent increase in the elastin promoter activity, which was maximally ~2.5-fold in the presence of 10 units/ml IL-1β (Table II). A similar effect of IL-1 was observed in transient transfections of fibroblast cultures established from the skin of donors of 21 and 73 years of age (not shown). It should be noted that the elastin promoter activity in these cells, as determined by the percent of acetylation of [14C]chloramphenicol, is relatively low, reflecting the low level of expression of the endogenous elastin gene in dermal fibroblasts, in agreement with previous observations from our laboratory (Kähari et al., 1990, 1992a). This observation contrasts the high level of elastin expression in cells derived from the lungs or arterial blood vessels, including aorta (Pierce et al., 1992; Hsu-Wong et al., 1992).

The effect of IL-1β on elastin gene expression at the transcriptional level was also examined in fibroblast cultures established from the skin or lungs of transgenic mice that express the human elastin promoter/CAT reporter gene incorporated into their germ line (Hsu-Wong et al., 1992). The expression of this construct has been shown to be tissue-specific, with the highest level of expression being noted in the lungs and aorta while the level of expression is relatively low in the skin. Incubation of the transgenic skin fibroblasts with IL-1β for 24 h resulted in a significant, up to 2.7-fold, increase in the promoter activity (Fig. 4). The maximum enhancement was noted with 10 units/ml IL-1β. Similarly, ~3.5-fold enhancement was observed in fibroblast cultures established from the lungs of transgenic animals when incubated with 10 units/ml IL-1β. It should be noted that in the transgenic cells, the elastin promoter is permanently integrated into the mouse genome, yet the response was similar to that noted in transient transfections of human cells.

Finally, we examined the effects of IL-1 on elastin gene expression in vivo, by subcutaneous injection of 100 units of recombinant human IL-1β into the transgenic mice described above. Low, yet clearly detectable levels of CAT activity were found in control skin extracts, contrasting with the high levels found in the lungs of these animals. CAT activity, when measured 30 h after the injection, was increased more than 4-fold in IL-1-treated animal skin versus control animal skin (Fig. 5). No systemic effect of IL-1 on elastin gene expression

![Image of Table II](image_url)

**Table II**

**Effect of human recombinant IL-1β on the elastin promoter activity in transient cell transfections**

Human neonatal fibroblasts were transfected with a human elastin promoter/CAT reporter gene construct, pEP1/CAT, together with a Rous sarcoma virus promoter/β-galactosidase reporter gene construct, as described under Materials and Methods. Three h after the glycerol shock, the cells were exposed to varying concentrations of IL-1β (0.1–10 units/ml) in medium containing 10% fetal calf serum. After 48 h of additional incubation, the cells were harvested and CAT activity was determined in duplicate cultures. Quantitation of CAT activity, as percent of the acetylated [14C]chloramphenicol expressed as the mean ± S.D. value of duplicate samples in a representative experiment. The values were corrected for β-galactosidase activity in the same samples, as an index of transfection efficiency. Also the promoterless construct BSOCAT was used in control transfections to estimate basal levels of chloramphenicol conversion, whereas pSVCAT, containing the SV40 promoter/enhancer, was used as a positive control.
transcriptional regulation of the elastin gene expression does occur, both in vitro and in vivo.

In summary, we have demonstrated that recombinant IL-1β up-regulates elastin gene expression in fibroblasts at the transcriptional level. This effect by IL-1β, together with the effects of other cytokines, such as transforming growth factor-β and tumor necrosis factor-α, attest to the possibility that the cytokine network can modulate elastin gene expression in a variety of inflammatory processes.

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REFERENCES

Chirgwin, J. M., Przybyla, A. E., McDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294-5299