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Interleukin-1β prevents the stimulatory effect of transforming growth factor-β on collagen gene expression in human skin fibroblasts

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Transforming growth factors β1 and β2 (TGF-β1 and TGF-β2) are well-characterized strong inducers of collagen gene expression. A 100 pM concentration of TGF-β1 or TGF-β2 increases proα1(I) collagen mRNA levels in human skin fibroblasts 6.6-fold and 7.0-fold respectively, and also increases the accumulation of procollagens in the cell culture medium. Interleukin-1β (IL-1β) is an inflammatory mediator which also regulates connective tissue metabolism. A small concentration of IL-1β (0.01–1.0 unit/ml) slightly increases proα1(I) collagen mRNA levels (2.2-fold). Here we provide evidence that IL-1β prevents the stimulatory effect of TGFs-β on collagen synthesis in human skin fibroblasts. An IL-1β concentration of 1 unit/ml is enough to keep proα1(I) collagen mRNA levels at control values in cells stimulated by 100 pM-TGF-β1. Thus the results indicate that IL-1β inhibits collagen synthesis in cells activated by TGFs-β, whereas it does not significantly change or might even stimulate collagen gene expression in non-activated cells.

INTRODUCTION

Transforming growth factors β1 (TGF-β1) and β2 (TGF-β2) are potent regulators of connective tissue metabolism (for reviews, see Sporn *et al.*, 1987; Massagué, 1990). TGFs-β increase the synthesis of extracellular matrix molecules including collagens (Ignotz & Massagué, 1986; Roberts *et al.*, 1986), fibronectin (Ignotz & Massagué, 1986), proteoglycans (Bassols & Massagué, 1988), tenascin (Pearson *et al.*, 1988), thrombospondin (Penttinen *et al.*, 1988), osteonectin (Noda & Rodan, 1987) and osteopontin (Noda *et al.*, 1988). They also decrease the degradation of matrix molecules by decreasing the synthesis of neutral metalloproteinases (NMPs; Matrisian *et al.*, 1986; Laiho *et al.*, 1986; Edwards *et al.*, 1987) and by increasing the synthesis of proteinase inhibitors (Laiho *et al.*, 1986; Edwards *et al.*, 1987). In addition to the induction of accumulation of the matrix components, TGFs-β increase cell adhesion to the extracellular matrix. TGFs-β stimulate the synthesis of several members of the integrin family, including receptors for type I collagen, fibronectin, vitronectin and laminin (Ignotz & Massagué, 1986; Heino *et al.*, 1989; Ignotz *et al.*, 1989). Also, studies *in vivo* suggest that TGFs-β are important in the regeneration of the connective tissue in wound healing and that they may also participate in fibrotic disorders (Roberts *et al.*, 1986).

Interleukin-1β (IL-1β) is an inflammatory mediator which may have an important role in tissue destruction in chronic inflammations (for review, see Dinarello, 1988). IL-1 increases the synthesis of NMPs, including collagenase, plasminogen activator and stromalysin (Mizel *et al.*, 1981; Mochan *et al.*, 1986; Murphy *et al.*, 1986). The degradation of extracellular matrix is probably the major effect of IL-1 on connective tissue. *In vitro*, this can be seen, for example, as a rapid loss of cell surface fibronectin in synovial fibroblasts activated by IL-1 (Heino, 1986). However, IL-1 can also regulate the synthesis of matrix molecules. It stimulates hyaluronan synthesis (Yaron *et al.*, 1987) and to a smaller extent also stimulates proteoglycan gene expression (Yaron *et al.*, 1987; Heino *et al.*, 1988). IL-1 has also been reported to stimulate collagen synthesis and increase procollagen mRNA levels (Goldring & Krane, 1987; Kähäri *et al.*, 1987; Postlethwaite *et al.*, 1988), but this effect seems to be dependent on the activation stage of the cells (Duncan & Berman, 1989). Furthermore, due to cell-line-specific and other, still poorly known, factors, IL-1 in many cell culture models decreases collagen production (Mauviel *et al.*, 1988; Harrison *et al.*, 1990). IL-1 injected into developing rat granulation tissue *in vivo* decreases collagen accumulation (Laato & Heino, 1988).

Here we have studied the effects of human recombinant IL-1β on collagen production in cultured human skin fibroblasts stimulated by TGFs-β, and we provide evidence that IL-1β is a potent antagonist to TGFs-β in the regulation of collagen synthesis.

MATERIALS AND METHODS

Human skin fibroblasts were cultured from skin samples obtained during surgery of an adult male without systemic diseases. Cells were cultured in Dulbecco's modification of Eagle's medium (Flow Laboratories, Irvine, Scotland, U.K.) supplemented with 10% fetal calf serum (Flow Laboratories). The cells used in the experiments were of the 8th passage.

Purified bovine bone TGF-β1 and -β2 were kindly provided by Dr. Joan Massagué (Sloan–Kettering Institute, New York, NY, U.S.A.). Human recombinant IL-1β was from Genzyme (Boston, MA, U.S.A.); 1 mg of IL-1 protein is equal to 10^8 units in the mouse thymocyte assay.

Cells were cultured to confluence and preincubated in the presence of 2% fetal calf serum, 50 μg of ascorbic acid/ml and various concentrations of IL-1β (0, 0.01, 0.1 and 1.0 unit/ml), with or without TGF-β1 or TGF-β2 (100 pM) for 24 h. After preincubation, the cells were placed in a similar fresh medium, which also contained 50 μg of β-aminopropionitrile (β-APN)/ml and 5 μCi of [^{3}H]proline/ml (sp. radioactivity 5.0 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.). After 24 h medium was collected and dialysed against water, and the amount of protein-bound [^{3}H]proline and [^{3}H]hydroxyproline was measured (Juva & Prockop, 1966). In this cell line, especially when cross-

Abbreviations used: β-APN, β-aminopropionitrile; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1β, interleukin-1β; NMP, neutral metalloproteinase; TGF-β, transforming growth factor-β.

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linking of collagen molecules is inhibited by β-APN, the amount of collagen in the cell layer is not significant compared with that in the medium.

Collagen in the cell culture medium was also measured by SDS/PAGE after a similar experiment. The medium was dialysed against 1 mM-ammonium carbonate buffer (pH 7.4), frozen and lyophilized. To degrade non-collagenous proteins, the samples were treated with pepsin (Jalkanen *et al.*, 1980). Type I and type III collagens were separated by interrupted electrophoresis (Sykes *et al.*, 1976). The polyacrylamide gels were prepared for autoradiography (Pulleyblank & Booth, 1981) and exposed to Kodak X-Omat films. Densitometric scans were used to quantify the collagen bands.

Isolation of total cellular RNA was performed as described previously (Chirgwin *et al.*, 1979). Portions (12 μg) of total cellular RNA were fractionated electrophoretically on a 0.75% agarose gel after denaturation with glyoxal, and transferred to a Pall Biodyne A nylon membrane (Pall Process Filtration Ltd., Portsmouth, U.K.). The membrane was hybridized using specific cDNA probes labelled by nick-translation with [α-^{32}P]dCTP (800 Ci/mmol; Amersham) to a specific radioactivity of approx. 5×10^7 c.p.m./μg. Plasmids used in hybridizations were pHCAL1, specific for human proα1(I) collagen (Vuorio *et al.*, 1987), and pRGAPDH13, specific for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Fort *et al.*, 1985). GAPDH is a 'house-keeping' enzyme, and its mRNA levels were measured as controls. Kodak X-Omat films were exposed to membranes, and the extent of hybridization was quantified densitometrically from the X-ray films.

RESULTS

TGF-β1 and -β2 are potent stimulators of type I collagen synthesis (Ignotz & Massagué, 1986). Here, 100 pM-TGF-β1 increased proα1(I) collagen mRNA levels in human skin fibroblasts by 6.6-fold (Fig. 1). A similar increase (7.0-fold) was also seen in 100 pM-TGF-β2-treated cells (results not shown). Accumulation of collagen in the cell culture medium during a 24 h period was measured using a [^{3}H]hydroxyproline assay (Table 1) and SDS/PAGE of pepsin-resistant proteins (Fig. 2). Incorporation of [^{3}H]proline into medium proteins was used to estimate the ratio of collagen/total protein. TGF-β1 increased the amount of type I collagen in the medium by 2.0-fold (Fig. 2) and [^{3}H]hydroxyproline by 1.4-fold (Table 1). The increases after TGF-β2 treatment were 3.0-fold (results not shown) and 2.2-fold (Table 1) respectively. The effect of TGFs-β on type III collagen was even smaller (Fig. 2). The increase in the accumulation of collagen was larger than that of total [^{3}H]proline-labelled protein (results not shown).

IL-1β (1 unit/ml) slightly increased proα1(I) collagen mRNA levels (2.2-fold, Fig. 1) but did not significantly increase the amount of collagen in the cell culture medium (Fig. 2, Table 1). However, in the presence of 0.01–1.0 unit of IL-1β/ml, TGF-β1 was a much less potent stimulator of collagen gene expression (Fig. 1). This effect of IL-1β was dose-dependent, and 1 unit/ml was sufficient to keep proα1(I) collagen mRNA at the control level (Fig. 1). IL-1β also inhibited the increase in proα1(I) collagen mRNA levels caused by TGF-β2 (results not shown). Furthermore, in cell cultures stimulated by TGFs-β, IL-1β inhibited the accumulation of type I and type III collagens and of [^{3}H]hydroxyproline into the cell culture medium in a dose-dependent manner (Table 1, Fig. 2). Thus in our experiments IL-1β was a potent antagonist to TGFs-β.

Table 1. Protein-bound [^{3}H]hydroxyproline in the cell culture medium

Cells were exposed to IL-1β and TGFs-β for 24 h and then labelled with [^{3}H]proline for another 24 h. Means ± S.E.M. of four samples are shown.

IL-1β (unit/ml)	[^{3}H]Hydroxyproline (d.p.m./1000 cells)		
	Control	+100 pM-TGF-β1	+100 pM-TGF-β2
0	89±2	125±5	198±17
0.01	92±5	124±4	168±1
0.1	88±6	119±8	179±12
1.0	83±6	90±4	137±6

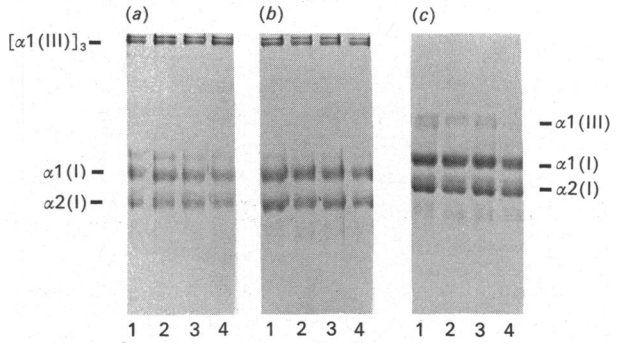

Fig. 2. SDS/PAGE of collagens in cell culture medium

Human skin fibroblasts were preincubated for 24 h in the presence of IL-1β (0–1.0 unit/ml) with (*b*, *c*) or without (*a*) 100 pM-TGF-β1 and then labelled for 24 h in the presence of [^{3}H]proline. Medium samples were collected and digested with pepsin. (*a*, *b*) SDS/PAGE in non-reducing conditions. Different type I collagen α-chains and triple-helical type III collagen are indicated. (*c*) Interrupted electrophoresis of the same samples showing α1(III) collagen. IL-β1 concentrations were: lane 1, control; lane 2, 0.01 unit/ml; lane 3, 0.1 unit/ml; lane 4, 1.0 unit/ml.

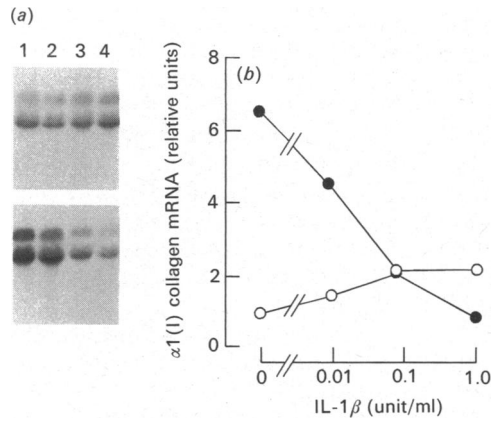

Fig. 1. Effects of IL-1β and TGF-β1 on proα1(I) collagen mRNA levels in human skin fibroblasts

Human skin fibroblasts were incubated for 24 h in the presence of various concentrations of IL-1β (lane 1, control; lane 2, 0.01 unit/ml; lane 3, 0.1 unit/ml; lane 4, 1.0 unit/ml) with (bottom) or without (top) 100 pM-TGF-β1. The monolayers were lysed, and total cellular RNA was obtained and subjected to Northern blot assays (*a*). The filter was also probed with a ^{32}P-labelled GAPDH cDNA, and the autoradiographic signal obtained with this probe was used to normalize the densitometric values used to generate plot (*b*). The units in plot (*b*) are relative to the mRNA level observed in untreated control cells. ○, IL-1β; ●, IL-1β + TGF-β1.

DISCUSSION

Destruction and regeneration of the extracellular matrix are phenomena regulated by growth and differentiation factors as well as by inflammatory mediators. Extracellular matrix molecules provide the integrity of tissues and organs, but they are also important regulators of cell differentiation, phenotype and metabolism (Ingber & Folkman, 1989). Furthermore, some effects of growth factors on cell behaviour might be mediated via changed cell adhesion (Ignotz & Massagué, 1987; Heino *et al.*, 1989; Heino & Massagué, 1989) or matrix composition (Nugent & Newman, 1989; Heino & Massagué, 1990).

The two polypeptide factors used in this study have opposite effects on the degradation of matrix components. IL-1β is a strong stimulator of the synthesis of NMPs (Mizel *et al.*, 1981 Mochan *et al.*, 1986; Murphy *et al.*, 1986), whereas TGFs-β decrease their production (Matrisian *et al.*, 1986; Laiho *et al.*, 1986; Edwards *et al.*, 1987). Recent studies have shown that TGFs-β also decrease proteinase synthesis in IL-1-activated cells (Chandrasekhar & Harvey, 1988; Lanyatis *et al.*, 1989). Furthermore, TGFs-β partially inhibit the autolysis of living cartilage by IL-1 (Andrews *et al.*, 1989). However, the effects of TGFs-β and IL-1β on collagen synthesis can be, at least in some experimental models, similar. Surprisingly, our data here show that in the same conditions as those in which IL-1β alone slightly increases proα1(I) collagen mRNA levels, this cytokine is a potent antagonist of TGF-β action on collagen synthesis. This supports the previous idea that the effect of IL-1 on collagen synthesis is dependent on the activation stage of the cells. Furthermore, given the relatively small concentration of IL-1β needed to block the effect of TGFs-β on collagen synthesis, this might be an important phenomenon in conditions *in vivo* where both factors are present. Interestingly, TGFs-β can increase IL-1 synthesis (Wahl *et al.*, 1987), and in this way may possibly inhibit their own action.

TGF-β1 stimulates collagen gene expression at the transcriptional level, at least partially via nuclear factor-1 (Rossi *et al.*, 1988). TGF-β1 can also increase collagen mRNA stability (Penttinen *et al.*, 1988). Here, as an effect of TGFs-β, proα1(I) collagen mRNA levels increased more than collagen accumulation into the media. Post-translational regulation of collagen gene expression by TGF-β has not been described, and we have not done experiments to address this question. The exact mechanism of IL-1 action on collagen synthesis is not known, but the regulation probably takes place at several levels. For example, IL-1 can increase procollagen mRNA levels while the actual procollagen protein synthesis decreases, suggesting the existence of both pre- and post-translational regulatory mechanisms (Mauviel *et al.*, 1990). A recent report studying the inhibition of collagen synthesis by IL-1 in osteoblastic MC3T3-E1 cells showed that IL-1 reduced the transcription of the α1(I) collagen gene (Harrison *et al.*, 1990). Our data show that IL-1β prevents the effect of TGFs-β on collagen gene expression at the mRNA level. However, further studies are needed to show whether this effect occurs via a transcriptional mechanism.

To conclude, several growth factors and cytokines take part in the regulation of collagen synthesis in different physiological and pathological conditions. TGFs-β are the most powerful stimulators of collagen synthesis known, but, as shown here, other factors can modify this effect.

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REFERENCES

Andrews, H. J., Edwards, T. A., Cawston, T. E. & Hazleman, B. L. (1989) Biochem. Biophys. Res. Commun. **162**, 144–150
Bassols, A. & Massagué, J. (1988) J. Biol. Chem. **263**, 3039–3045
Chandrasekhar, S. & Harvey, A. K. (1988) Biochem. Biophys. Res. Commun. **157**, 1352–1359
Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) Biochemistry **18**, 5294–5299
Dinarello, C. A. (1988) FASEB J **2**, 108–115
Duncan, M. R. & Berman, B. (1989) J. Invest. Dermatol. **92**, 699–706
Edwards, D. R., Murphy, G., Reynolds, J. J., Whitman, S. E., Docherty, A. J. P., Angel, P. & Heath, J. K. (1987) EMBO J. **6**, 1899–1904
Fort, P., Marty, L., Piechaczyk, M., El Sabrouty, S., Dani, C., Jeanteur, P. & Blanchard, J. M. (1985) Nucleic Acids Res. **13**, 1431–1442
Goldring, M. B. & Krane, S. M. (1987) J. Biol. Chem. **262**, 16724–16729
Harrison, J. R., Vargas, S. J., Petersen, D. N., Lorenzo, J. A. & Kream, B. E. (1990) Mol. Endocrinol. **4**, 184–190
Heino, J. (1986) Virchows Arch. (Cell Pathol.) **50**, 313–320
Heino, J. & Massagué, J. (1989) J. Biol. Chem. **264**, 21806–21811
Heino, J. & Massagué, J. (1990) J. Biol. Chem. **265**, 10181–10184
Heino, J., Kähäri, V.-M., Mauviel, A. & Krusius, T. (1988) Biochem. J. **252**, 309–312
Heino, J., Ignotz, R., Hemler, M., Crouse, C. & Massagué, J. (1989) J. Biol. Chem. **264**, 380–388
Ignotz, R. A. & Massagué, J. (1986) J. Biol. Chem. **261**, 4337–4345
Ignotz, R. A. & Massagué, J. (1987) Cell **48**, 549–554
Ignotz, R., Heino, J. & Massagué, J. (1989) J. Biol. Chem. **264**, 389–392
Ingber, D. E. & Folkman, J. (1989) Cell **58**, 803–805
Jalkanen, M., Tammi, M., Vihersaari, T., Peltonen, J., Kulonen, E. & Penttinen, R. (1980) J. Biophys. Methods **2**, 331–339
Juva, K. & Prockop, D. (1966) Anal. Biochem. **15**, 77–83
Kähäri, V.-M., Heino, J. & Vuorio, E. (1987) Biochim. Biophys. Acta **929**, 142–147
Laato, M. & Heino, J. (1988) Experientia **44**, 32–34
Laiho, M., Saksela, O., Andreasen, P. A. & Keski-Oja, J. (1986) J. Cell Biol. **103**, 2403–2410
Lanyatis, R., Thompson, N. L., Remmers, E. F., Flanders, K. C., Roche, N. S., Kim, S.-J., Case, J. P., Sporn, M. B., Roberts, A. B. & Wilder, R. L. (1989) J. Immunol. **143**, 1142–1148
Massagué, J. (1990) Annu. Rev. Cell Biol. **6**, in the press
Matrisian, L. M., Leroy, P., Ruhlmann, C., Gesnel, M.-C. & Breathnach, R. (1986) Mol. Cell. Biol. **6**, 1679–1686
Mauviel, A., Teyton, L., Bhatnagar, R., Penfornis, H., Laurent, M., Hartmann, D., Bonaventure, B., Loyau, G., Saklatvala, J. & Pujol, J.-P. (1988) Biochem. J. **252**, 247–255
Mauviel, A., Heino, J., Kähäri, V.-M., Hartmann, D. J., Loyau, G., Pujol, J.-P. & Vuorio, E. (1990) J. Invest. Dermatol., in the press
Mizel, S. B., Dayer, J.-M., Krane, S. M. & Mergenhagen, S. E. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 2474–2477
Mochan, E., Uhl, J. & Newton, R. (1986) J. Rheumatol. **13**, 15–19
Murphy, G., Hembry, R. M. & Reynolds, J. J. (1986) Collagen Relat. Res. **6**, 351–364
Noda, M. & Rodan, G. A. (1987) J. Cell. Physiol. **133**, 426–437
Noda, M., Yoon, K., Prince, C. W., Butler, W. T. & Rodan, G. A. (1988) J. Biol. Chem. **263**, 13916–13921
Nugent, M. A. & Newman, M. J. (1989) J. Biol. Chem. **264**, 18060–18067
Pearson, C. A., Pearson, D., Shibahara, S., Hofsteenge, J. & Chiquet-Ehrismann, R. (1988) EMBO J. **7**, 2677–2681
Penttinen, R. P., Koyayashi, S. & Bornstein, P. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 1105–1108
Postlethwaite, A. E., Raghow, R., Stricklin, G. P., Poppleton, H., Seyer, J. M. & Kang, A. H. (1988) J. Cell Biol. **106**, 311–318
Pulleyblank, D. E. & Booth, G. H. (1981) J. Biochem. Biophys. Methods **4**, 339–346
Roberts, A. B., Sporn, M. B., Assoian, R. K., Smith, J. M., Roche, N. S., Wakefield, L. M., Heine, U. I., Liotta, L. A., Falanga, V., Kehrl, J. H. & Fauci, A. S. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 4167–4171
Rossi, P., Karsenty, G., Roberts, A. B., Roche, N. S., Sporn, M. B. & de Crombrugghe, B. (1988) Cell **52**, 405–414
Sporn, M. B., Roberts, A. B., Wakefield, L. M. & de Crombrugghe, B. (1987) J. Cell Biol. **105**, 1039–1045

Sykes, B., Puddle, B., Francis, D. & Smith, R. (1976) Biochem. Biophys. Res. Commun. **72**, 1472–1480

Vuorio, T., Mäkelä, J. K., Kähäri, V.-M. & Vuorio, E. (1987) Arch. Dermatol. Res. **279**, 154–160

Wahl, S. M., Hunt, D. A., Wakefield, L. M., McCartney-Francis, N., Wahl, L. M., Roberts, A. B. & Sporn, M. B. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 5788–5792

Yaron, I., Meyer, F. A., Dayer, J.-M. & Yaron, M. (1987) Arthritis Rheum. **30**, 424–430

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