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Leukoregulin, A T-Cell Derived Cytokine, Upregulates Stromelysin-1 Gene Expression in Human Dermal Fibroblasts: Evidence for the Role of AP-1 in Transcriptional Activation

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Abstract Leukoregulin (LR), a product of activated T-cells, has been recently shown to modulate the metabolism of extracellular matrix components in human skin fibroblast cultures (Mauviel et al., J Cell Biol 113:1455–1462, 1991). In this study we focused our attention on the effects of LR on the expression of stromelysin-1 gene. This matrix metalloprotease has a broad spectrum of degradative activity and it is also required for maximal activation of interstitial collagenase. Incubation of skin fibroblast cultures with LR resulted in a dose- and time-dependent elevation of stromelysin-1 mRNA levels, the maximum enhancement being up to approximately sevenfold. This effect was abolished by cycloheximide, suggesting a requirement for ongoing protein synthesis. Transient cell transfections with a promoter/reporter gene construct containing 1.3 kb of 5′ flanking DNA of the human stromelysin-1 gene linked to the chloramphenicol acetyl transferase (CAT) gene, indicated enhancement of promoter activity by LR. This enhancement was abolished by a single base substitution in the AP-1 binding site of the promoter. Furthermore, gel mobility shift assays demonstrated enhanced AP-1 binding activity in nuclear extracts from cells incubated with LR. However, LR did not alter the activity of a construct containing three AP-1 sequences in front of the thymidine kinase promoter linked to the CAT gene. These results collectively suggest that activation of stromelysin-1 gene expression by LR is mediated by AP-1 regulatory elements which are necessary, but not sufficient, for gene response.

Key words: leukoregulin, human dermal fibroblasts, stromelysin, transcription, T-cells

Matrix metalloproteinases (matrixins) comprise a family of proteolytic enzymes involved in the degradation of the extracellular matrix of connective tissue. These enzymes play a critical role in tissue development, homeostasis, and repair, as well as in pathological degradation of the extracellular matrix in diseases [Krane et al., 1990; Woessner, 1991]. One of the members of this family is stromelysin-1 (also known as MMP-3 or proteoglycanase) which has a relatively broad spectrum of proteolytic activity and can degrade proteoglycan link protein, fibronectin, and laminin, as well as various gelatins and collagens [Krane et al., 1990; Woessner, 1991]. Furthermore, stromelysin-1 is required for maximal activation of collagenase by cleaving the precursor molecule, procollagenase, to its active form [Brinckerhoff et al., 1990]. Thus, stromelysin-1 plays a major role in different aspects of the extracellular matrix degradation.

Recent studies have indicated that a variety of cytokines and growth factors are capable of modulating the production of matrix metalloproteinases by different cell types [Krane et al., 1990].

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One of them is a recently discovered cytokine, leukoregulin (LR), a ~50 kD glycoprotein which is produced by activated T-cells [Ransom et al., 1985]. This cytokine was initially shown to have unique anti-proliferative effects on tumor cells, and it was shown to produce a rapid and reversible increase in the permeability of plasma membranes of the target cells [Barnett and Evans, 1986; Evans, 1987; Evans et al., 1987]. More recently, we have shown that LR can modulate extracellular matrix metabolism by normal human fibroblasts in vitro. Specifically, it was demonstrated that LR can down-regulate the synthesis and secretion of type I and type III collagens and fibronectin, while the production of hyaluronic acid and several metalloproteases was shown to be increased [Mauviel et al., 1991]. Furthermore, LR was shown to elicit time- and dose-dependent induction of collagenase mRNA steady-state levels, and this effect was shown to be dependent on active protein synthesis [Mauviel et al., 1992]. Transient transfections of cultured fibroblasts with a human collagenase promoter/reporter gene construct indicated up-regulation of the promoter activity, suggesting regulation at the transcriptional level of collagenase gene expression [Mauviel et al., 1992]. Consequently, the ability of LR to induce collagenase gene expression in skin fibroblasts suggested that this cytokine may contribute to the degradation of the extracellular matrix both in physiological as well as in pathological situations.

To further elucidate the role of LR in the degradation of extracellular matrix, we have now examined the effects of this cytokine on stromelysin-1 gene expression. The results indicate up-regulation of stromelysin-1 gene expression, as detected at the mRNA level. Transient cell transfections and gel retardation assays suggest transcriptional activation involving the AP-1 regulatory element known to reside in the promoter region of this gene.

MATERIALS AND METHODS

Cell Cultures

Adult human skin fibroblast cultures, established from tissue specimens obtained from cosmetic surgery procedures, and neonatal foreskin fibroblast cultures were utilized in passages 3–8. The cell cultures were maintained in Dulbecco's modified Eagle's (DME) medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 50 μg/ml streptomycin. Mouse NIH-3T3 cells were purchased from the American Type Culture Collection (Rockville, MD) and maintained in DME medium supplemented with 10% calf serum, 2 mM glutamine, and the antibiotics indicated above.

Preparation of Leukoregulin

LR with a pI of 5.1 and a molecular mass of ~50 kD, free of IL-1α, IL-1β, IFN-γ, lymphotoxin, and TNF-α [Ransom et al., 1985; Mauviel et al., 1991], was purified from human peripheral blood leukocytes as previously described [Evans et al., 1989]. Briefly, normal human lymphocytes were stimulated with phytohaemagglutinin (leukoagglutinin isomer; Sigma Chemical Co., St. Louis, MO) for 48 h, and LR was purified using sequential diafiltration, anion exchange, isoelectric focusing, and high performance molecular sieving liquid chromatography. One unit (U) of LR was defined as the amount of activity causing 50% increase in the plasma membrane permeability of K562 erythroleukemia cells, 1×10^6 cells per milliliter, during a 2 h incubation [Barnett and Evans, 1986].

Northern Analyses

Confluent human skin fibroblast cultures incubated with or without LR were subjected to isolation of total cellular RNA, as described previously [Chirgwin et al., 1979]. In some cultures, dexamethasone (DEX) or all-*trans*-retinoic acid (RA), both in 10^{-6} M concentration, were added 1 h prior to the addition of LR. Total RNA, 10–20 μg per lane, was fractionated on 0.8% agarose gels, transferred to nylon filters (Zeta Probe, BioRad Laboratories, Richmond, CA), and immobilized by heating at 80°C for 30 min under vacuum. The filters were then prehybridized and hybridized with cDNA probes labeled by nick translation with both [α-^{32}P]dGTP and [α-^{32}P]dCTP [Thomas, 1980; Sambrook et al., 1989]. The [^{32}P]cDNA-mRNA hybrids were visualized by autoradiography, and the corresponding steady-state levels of mRNA were quantitated by scanning densitometry using a He-Ne laser scanner at 633 nm (LKB Produkter, Bromma, Sweden).

The following cDNAs were used for Northern hybridizations: for stromelysin-1, a 1.5 kb human cDNA [Saus et al., 1988]; for collagenase, a 2.0 kb human cDNA [Goldberg et al., 1986]; and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, a 1.3 kb rat cDNA (pRGAPDH13) [Fort et al., 1985]. To perform rehybridizations with

another cDNA probe, the Northern filters were boiled in 0.1 × SSC containing 0.5% SDS, twice for 5 min. The filters were then prehybridized and hybridized as indicated above.

Transient Transfections of Cultured Cells

Human foreskin fibroblasts in late logarithmic growth phase were transfected with 10 or 20 μg of construct DNA 4+CAT, which contains 1.3 kb of 5′-flanking DNA of human stromelysin-1 gene linked to the CAT reporter gene [Buttice et al., 1991]. This construct has been shown to contain an AP-1 binding site, TGAGTCA, in the position −70 to −64. Parallel transfections were performed with the M1CAT construct, which contains the same 5′-flanking region as 4+CAT, but the putative AP-1 binding site has been mutated to GGAGTCA, and thus it does not bind Jun-Fos (AP-1) protein complexes [Buttice et al., 1991]. Additional transfections were performed with a construct, pAPCAT2a, which contains three AP-1 consensus sequences in front of the thymidine kinase promoter in pBLCAT2 plasmid [Frisch et al., 1990]. A RSV promoter/β-galactosidase reporter gene construct was co-transfected in every experiment, and β-galactosidase activity present in the cells at the end of the incubation period was used as a control of transfection efficiency [Sambrook et al., 1989]. 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 1% or 10% heat-inactivated fetal calf serum. After 3 h of incubation, LR (1 U/ml) was added, and the incubations were continued for 40 h. The cells were then harvested and lysed by three cycles of freeze-thawing in 100 μl of 0.25 M Tris-HCl, pH 7.8. The protein concentration of each extract was determined with a protein assay kit (BioRad Laboratories, Richmond, CA), and identical amounts (5–10 μg per assay) of protein from each cell extract were used for parallel determinations of CAT activity using [^{14}C]chloramphenicol as substrate [Gorman et al., 1982]. The acetylated and non-acetylated forms of radioactive chloramphenicol were separated by thin-layer chromatography and visualized by autoradiography. The enzyme activity was quantified by cutting out pieces of thin-layer chromatography plates containing different forms of [^{14}C]chloramphenicol converted to its acetylated forms, after correction for β-galactosidase activity in each cell extract.

Gel Mobility Shift Assay

For gel retardation assays, nuclear proteins were isolated from neonatal foreskin fibroblasts, which had been incubated without or with LR for 5 h, using a small scale preparation technique (Andrews and Faller, 1991). For DNA binding assay, a double-stranded oligomer containing the collagenase/stromelysin-1 AP-1 binding site (underlined) was used: 5′-CTAGTGA-TGAGTCAGCCGGATC-3′. The end-labeled oligomer (total radioactivity per reaction, 3×10^4 cpm) was incubated with 5–10 μg of the protein extract for 30 min on ice in a total volume of 20 μl, as described previously [Dignam et al., 1983]. As a competitor for the binding, a hundredfold molar excess of the same oligomer was added to the binding reaction. The unbound oligomer and the DNA-protein complexes were then separated on a 4% polyacrylamide gel in 0.4 × TBE. The gels were dried and exposed to X-ray film at −70°C overnight.

RESULTS

Demonstration That LR Enhances Stromelysin-1 mRNA Steady-State Levels in Skin Fibroblasts

To examine the effects of LR on stromelysin-1 gene expression, human skin fibroblasts in culture were first incubated with LR in various concentrations for 24 h, and stromelysin-1 mRNA levels were determined by Northern hybridizations. The results, as shown in Figure 1, demonstrated that in control cultures, a faint, yet clearly detectable mRNA band with the apparent size of ~2.0 kb was detected with the stromelysin-1 cDNA. Addition of LR to the incubation medium resulted in a dose-dependent increase in the relative mRNA levels, the increase in the presence of 2 U/ml of LR being in this experiment approximately threefold, after the mRNA levels were corrected for the GAPDH mRNA abundance in the same RNA preparations (Fig. 1A). To examine the time-dependence of the LR-induced up-regulation of stromelysin-1 gene expression, fibroblast cultures were incubated in the presence of 1 U/ml of LR for varying time periods up to 48 h (Fig. 1B). Relatively little, if any, change was noted during the first 6 h of incubation, while at the 24 h time point, a ~4.5-fold increase was noted. Further incubation of cells up to 48 h revealed up to an

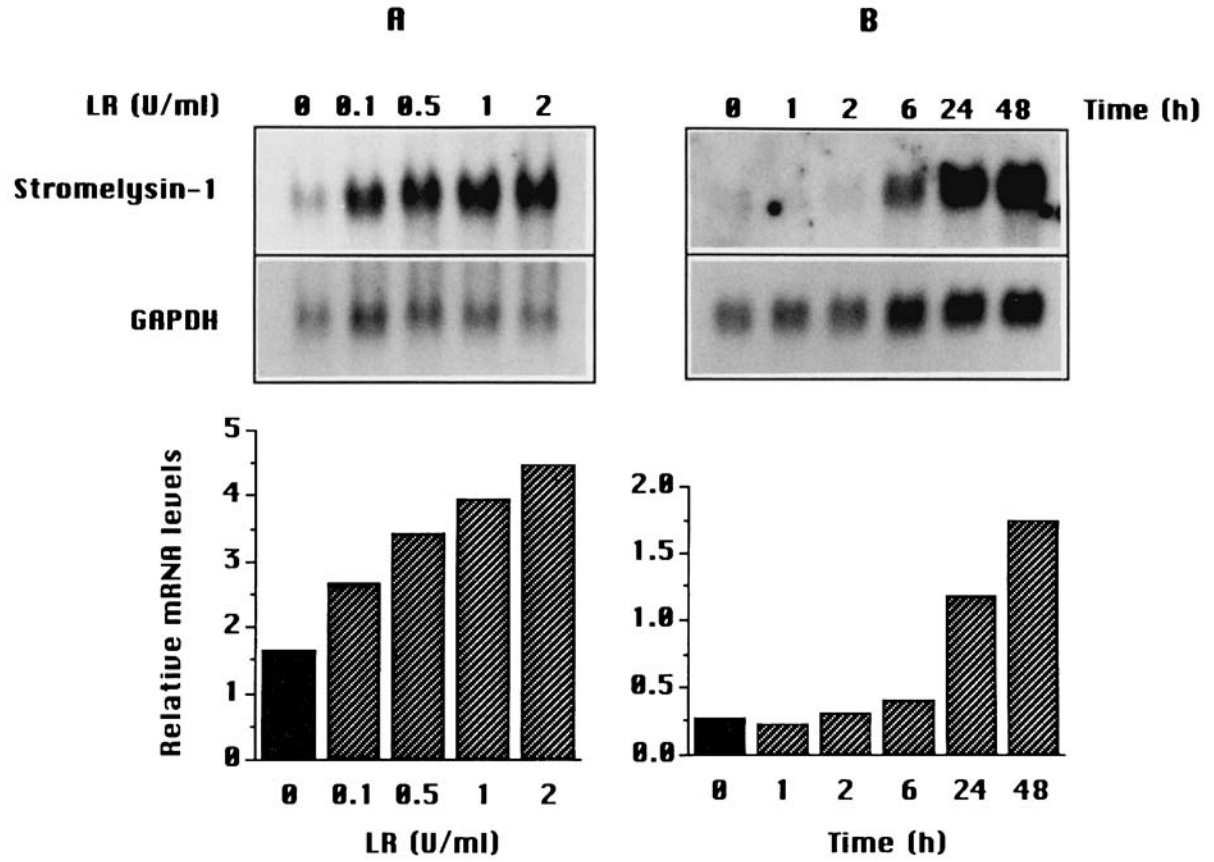

Fig. 1. Induction of stromelysin-1 mRNA steady-state levels in human skin fibroblasts by LR. **A:** LR-dose response. Confluent cultures of human skin fibroblasts were incubated with varying concentrations of LR (0.1-2 U/ml) for 24 h in medium containing 10% FCS. Total RNA (20 μg/lane) was analyzed by Northern hybridizations with cDNA probes for stromelysin-1 and GAPDH. **B:** Time-course experiment. Confluent fibroblast cultures were incubated with LR (1 U/ml) for up to 48 h in medium containing 10% FCS. Total RNA (12 μg/lane) was extracted at different time points and analyzed by Northern hybridizations, as above.

approximately sevenfold increase in stromelysin-1 mRNA levels, as compared to the control cultures incubated in parallel without LR. Thus, LR demonstrated both dose- and time-dependent up-regulation of stromelysin-1 mRNA levels.

To determine whether the up-regulation of stromelysin-1 gene expression by LR requires ongoing protein synthesis, cycloheximide (CHX) was added to some cultures either alone or 1 h prior to the addition of LR (1 U/ml), for a total of 24 h of incubation. As indicated in Figure 2, LR elicited a significant increase in stromelysin-1 mRNA levels. Incubation of cells with cycloheximide (10 μg/ml) alone resulted in an approximately sevenfold increase in the stromelysin-1 mRNA levels, confirming a previous report [Otani et al., 1990]. In addition, cycloheximide completely blocked the up-regulation elicited by LR. Thus, LR elicits its effects on stromelysin-1 mRNA levels by a process dependent on active protein synthesis.

Dexamethasone and All-*Trans*-Retinoic Acid Counteract LR-Elicited Up-Regulation of Stromelysin-1 mRNA Levels

Since previous studies have clearly demonstrated that dexamethasone (DEX) and all-*trans*-retinoic acid (RA) are capable of down-regulating metalloproteinase gene expression in human skin fibroblasts [Brinckerhoff et al., 1986; Frisch and Ruley, 1987; Brinckerhoff and Auble, 1990], we examined the possibility that these effector molecules could counteract LR-elicited up-regulation of stromelysin-1 gene expression. As shown in Table I, the relative mRNA levels for stromelysin-1 were increased in this experiment by ~5.5-fold by LR (1 U/ml). This increase was essentially abolished by the addition of DEX (10^{-6} M) and partially counteracted by RA (10^{-6}

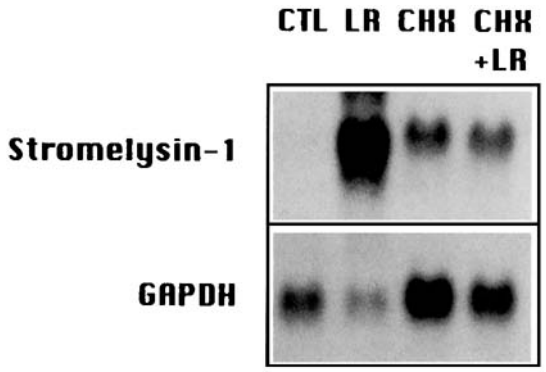

Fig. 2. Cycloheximide abrogates the LR-induced up-regulation of stromelysin-1 gene expression. Confluent fibroblast cultures were incubated in medium containing 1% FCS with or without LR (1 U/ml) which was added 1 h after the addition of cycloheximide (CHX) (10 μg/ml). Total RNA (10 μg/lane) was extracted after a 24 h incubation and analyzed by Northern hybridizations with stromelysin-1 and GAPDH cDNAs.

TABLE I. Demonstration That Dexamethasone and All-*Trans*-Retinoic Acid Counteract the LR-Induced Up-Regulation of Stromelysin-1 and Collagenase Gene Expression*

	Treatment of cells			
mRNA	CTL	LR	LR + DEX	LR + RA
Stromelysin-1	1.59	8.78	2.14	3.83
	(1.0)	(5.5)	(1.4)	(2.4)
Collagenase	1.56	30.8	4.83	11.1
	(1.0)	(19.7)	(3.1)	(7.1)

*Dexamethasone (DEX) or retinoic acid (RA), both in 10^{-6} M concentration, were added to confluent human adult skin fibroblast cultures maintained in medium containing 1% FCS. One hour later, LR (1 U/ml) was added as indicated. Total RNA was extracted after a 24 h incubation and analyzed by Northern hybridizations with stromelysin-1, collagenase, and GAPDH cDNAs. The autoradiograms were quantitated by scanning densitometry and the values for stromelysin-1 and collagenase mRNAs were corrected for GAPDH mRNA levels. The values are expressed as densitometric units, and the numbers in parentheses indicate -fold induction by the treatment in relation to untreated control cultures (CTL).

M) (Table I). Parallel examination of collagenase mRNA steady-state levels in the same blots, as detected by re-hybridization of the filters with a human collagenase cDNA, revealed a similar pattern, although the enhancement of collagenase gene expression by LR alone was considerably higher than that noted in case of stromelysin-1 (Table I). This stronger enhancement of collagenase mRNA levels as compared to those for stromelysin-1 was observed in four out of five independent experiments. Similar selective enhancement of collagenase mRNA levels, in comparison to stromelysin-1, by IL-1β has been recently reported in normal skin fibroblasts [Nguyen et al., 1992]. Nevertheless, these results suggest the presence of similar regulatory pathways for modulation of the expression of both collagenase and stromelysin-1 genes by LR.

Demonstration That LR Enhances Stromelysin-1 Promoter Activity

To examine the mechanisms leading to enhancement of stromelysin-1 mRNA steady-state levels by LR, transient cell transfections with a stromelysin-1 promoter/reporter gene construct were performed. The construct, 4+CAT, consisted of 1.3 kb of 5′-flanking region of stromelysin-1 gene linked to the chloramphenicol acetyl transferase (CAT) reporter gene. Neonatal human dermal fibroblasts were transfected and the cultures were then treated with LR (1 U/ml) in medium containing 10% FCS, as indicated in the Methods and in Figure 3. Assay of CAT activity after 40 h of incubation indicated that LR enhanced the promoter activity by approximately three- to fourfold, after correction by β-galactosidase activity in the same cell extracts (Fig. 3A). When the cell transfection experiments were performed in the presence of 1% FCS, the basal promoter activity in the control cultures was reduced to about 25% of the values in the presence of 10% FCS. However, essentially identical enhancement of the promoter activity by LR could be noted (results not shown). Thus, the enhancement of stromelysin-1 gene expression, as detected at the mRNA level, appears to be mediated, at least in part, by transcriptional activation of the promoter of the gene.

Evidence for the Role of AP-1

Regulation of stromelysin-1 gene expression by cytokines and phorbol esters, such as IL-1 and TPA, has been shown to involve AP-1 [Angel et al., 1987; Buttice et al., 1991; Sirum-Connolly and Brinckerhoff, 1991]. We have recently shown that LR induces expression of the gene for *jun*-B, a potential member of the AP-1 complex [Mauviel et al., 1992]. In this study, we examined the role of AP-1 in LR-elicited up-regulation of stromelysin-1 gene expression. First, transient cell transfections were performed with a mutant construct, M1CAT, which

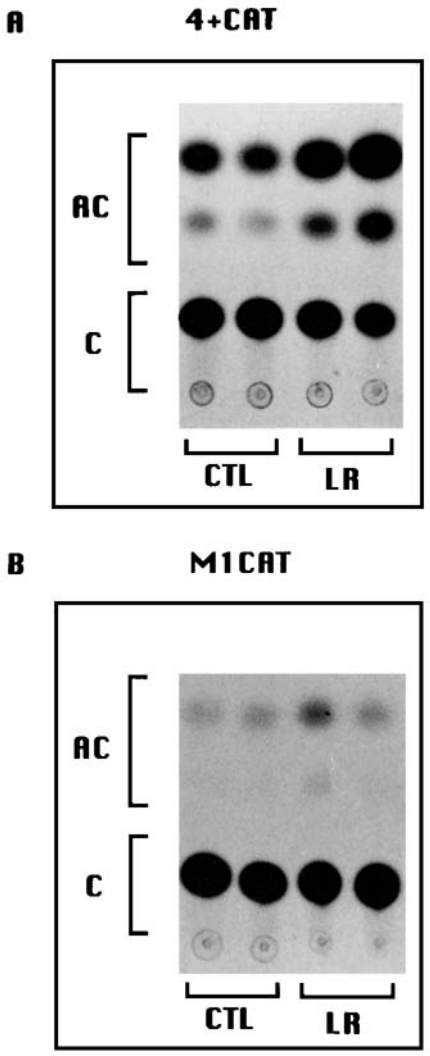

Fig. 3. Enhancement of stromelysin-1 promoter activity by LR in transient cell transfections, and evidence for the role of an AP-1 *cis*-element. Human neonatal foreskin fibroblasts were transfected with the human stromelysin-1 promoter/CAT reporter gene constructs 4+CAT or M1CAT, as described in Materials and Methods. Three hours after the glycerol shock, the cells were exposed to LR (1 U/ml) in medium containing 10% FCS. Following 40 h of additional incubation, the cells were harvested and CAT activity was determined. The figure shows the CAT assay depicting separation of acetylated (AC) and unacetylated (C) forms of [^{14}C]chloramphenicol by thin-layer chromatography. **A:** Cells were transfected with the 4+CAT construct containing 1.3 kb of the 5′-flanking region of the human stromelysin-1 gene. **B:** Cells were transfected in parallel cultures with the M1CAT construct which contains the same 1.3 kb promoter sequences as the 4+CAT construct but the AP-1 site at position −70 to −64 was mutated by site-directed mutagenesis (see text).

contains the same 1.3 kb fragment of the 5′-flanking region of stromelysin-1 promoter DNA as the construct 4+CAT, but the putative AP-1 binding site, TGAGTCA, has been mutated by site-directed mutagenesis. The mutated sequence, GGAGTCA, in the M1CAT construct does not bind Jun-Fos complexes of the AP-1 *trans*-regulatory elements [Buttice et al., 1991].

Transfection of human skin fibroblasts with M1CAT, followed by incubation with 1 U/ml of LR, indicated that the induction by LR was only ~1.4-fold, as compared to the induction of approximately three- to fourfold noted with the construct 4+CAT in a parallel transfection experiment (Fig. 3). These results suggested that the AP-1 binding site is involved in LR-elicited up-regulation of stromelysin-1 gene expression. It should be noted that the basal level of expression of M1CAT was consistently lower than that of 4+CAT. As shown in Figure 3, the CAT expression in M1CAT transfected control cells was only about 20% of that noted in cultures transfected with 4+CAT. Thus, the AP-1 binding site present in the promoter of the stromelysin-1 gene appears to play a role also in the basal expression of the gene. This representative experiment was performed in the presence of 10% FCS, and similar results were obtained in another experiment under identical conditions, as well as in two additional experiments in the presence of 1% FCS (not shown).

The role of AP-1 in these processes was further examined by gel mobility shift assays. In these experiments, nuclear extracts from neonatal fibroblasts incubated for 5 h with or without LR (1 U/ml) in medium containing 10% FCS were incubated with an AP-1 oligonucleotide radioactively labeled by ^{32}P. This 23 bp AP-1 oligonucleotide contains a consensus AP-1 binding site previously identified in the collagenase and stromelysin-1 promoters [Angel et al., 1987; Quinones et al., 1989]. Examination of the DNA-protein complexes by non-denaturing polyacrylamide gel electrophoresis indicated that the proteins isolated from control cultures resulted in the formation of a DNA-protein complex which was present at low, yet clearly detectable level (Fig. 4). Incubation of cells with LR markedly enhanced the binding activity of proteins detected in this band, and in addition, three other lower abundance DNA-protein complexes were noted (Fig. 4). The latter binding was largely abolished by incubation with a hundredfold excess of unlabeled AP-1 oligonucleotide but could not be competed by a hundredfold excess of NF-1–containing 20 bp oligomer, suggesting specificity of the binding to AP-1. Collectively, these observations suggest that the AP-1 com-

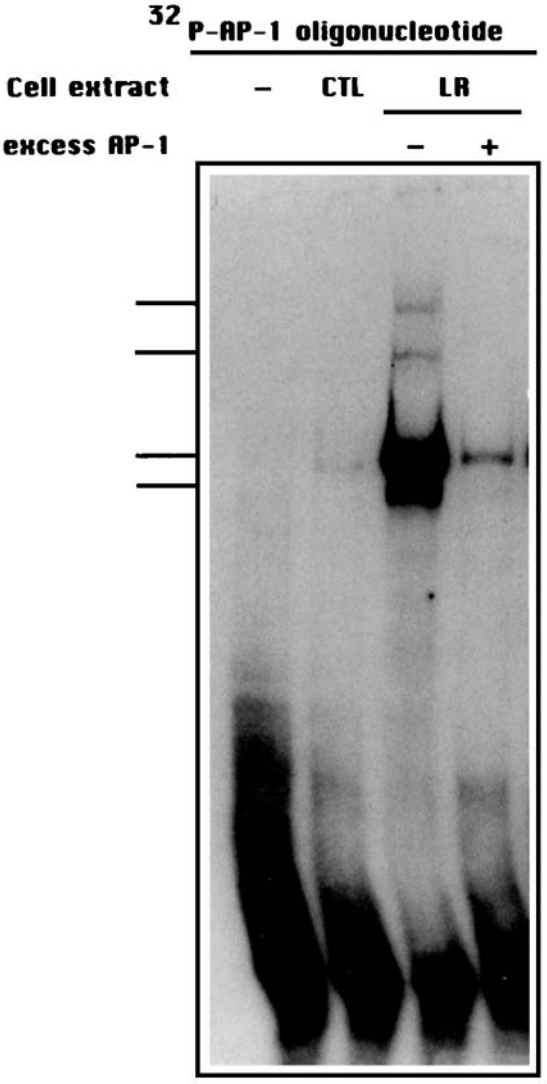

Fig. 4. Demonstration that LR enhances nuclear protein binding to the collagenase/stromelysin-1 AP-1 *cis*-elements, as analyzed by gel mobility shift assay. A radioactively labeled double-stranded oligonucleotide, 5′-CTAGTGATGAGTCAGCCGGATC-3′, containing the AP-1 binding sequence (underlined), was incubated with nuclear extracts from foreskin fibroblasts treated with or without LR (1 U/ml) for 5 h in medium containing 10% FCS. The reaction mixture was subsequently fractionated by electrophoresis on 4% nondenaturing polyacrylamide gels. Competition assay was performed with a hundredfold molar excess of unlabeled AP-1–containing oligonucleotide.

plex plays a role in the transcriptional activation of stromelysin-1 gene expression by LR.

To test whether the AP-1 *cis*-element alone was sufficient to mediate the LR-response, transient cell transfections were performed with a construct, pAPCAT2a, which contains three AP-1 consensus sequences in front of the thymidine kinase promoter linked to the CAT gene. Transfections of neonatal skin fibroblasts revealed a strong promoter activity, but this activity was not modulated by the addition of LR (1 U/ml) to the culture medium (results not shown). Thus, these results, taken together with transfections with the M1CAT construct (Fig. 3) and gel mobility shift assays (Fig. 4), suggest that the AP-1 binding site is necessary but not sufficient for the induction of stromelysin-1 gene expression by LR.

DISCUSSION

A variety of effector molecules, including several cytokines and growth factors, have been previously shown to modulate the expression of matrix metalloproteinase genes [Krane et al., 1990; Woessner, 1991]. One of the early demonstrations involved TPA which was shown to increase the expression of collagenase and stromelysin-1 genes [Frisch and Ruley, 1987; Angel et al., 1987]. It has also been demonstrated that this increase is mediated by binding of the AP-1 complexes to AP-1 *cis*-elements in the corresponding promoters [Angel et al., 1987; Quinones et al., 1989; Otani et al., 1990]. Similarly, monocyte derived factors, such as IL-1 and TNF-α, have been shown to be potent activators of matrix metalloprotease gene expression [Brenner et al., 1989; Conca et al., 1989]. This stimulation is accompanied by the expression of cellular proto-oncogenes, but the specific pattern of expression is somewhat variable depending on the cytokine. For example, incubation of human fibroblasts with TNF-α was shown to stimulate collagenase gene transcription accompanied by activation of *jun* gene expression, the major component of the AP-1 complex [Brenner et al., 1989]. This induction of *jun* mRNAs lasted for several hours, but by 24 h the levels had returned to the basal level. In contrast, TPA treatment resulted in a more transient increase in the *jun* mRNA levels, the maximum stimulation being noted after 1 h of incubation [Brenner et al., 1989]. It has also been demonstrated that IL-1 is able to enhance the synthesis and secretion of collagenase, and we have recently demonstrated that this induction parallels enhanced expression of c-*jun* and *jun*-B in skin fibroblast cultures [Mauviel et al., 1992]. Thus, these results suggest that AP-1 *cis*-element is involved in the up-regulation of collagenase gene expression.

In contrast to cytokine stimulation of metalloprotease gene expression, transforming growth factor-β1 has been shown to reduce collagenase activity in cell cultures. This inhibition appears to result from two distinct mechanisms: first,

TGF-β reduces the expression of the collagenase gene, and secondly, the expression of tissue inhibitor of metalloproteinase-1 (TIMP) is elevated [Edwards et al., 1987]. It should be noted that TIMP is an inhibitor of both collagenase and stromelysin-1 activity [Woessner, 1991]. TGF-β1 has also been shown to inhibit stromelysin-1 gene expression, and recent results suggest that this inhibition is mediated by the binding of a Fos-containing protein complex to the TGF-β inhibitory element (TIE) present in stromelysin-1 promoter sequences [Kerr et al., 1990]. Another study has suggested, however, that the transcriptional activation of collagenase gene expression by oncogenes and phorbol esters requires the *fos* gene expression [Schönthal et al., 1988]. Thus, the regulation of matrix metalloproteases appears to be largely transcriptionally controlled by complex mechanisms involving *cis*- and *trans*-acting regulatory elements, including members of the Fos and Jun families of oncogenes.

In the present study, we have tested the effects of a recently described cytokine, leukoregulin, on the expression of stromelysin-1 gene in human skin fibroblasts. The results demonstrated an approximately three- to fivefold activation of stromelysin-1 gene expression, as determined both at the mRNA steady-state level and by transient transfections of the cells with stromelysin-1 promoter/CAT construct. The concordance of the enhancement, as detected by these two different types of assays, suggests that stromelysin-1 gene expression by LR is primarily regulated at the transcriptional level. It should be noted that parallel examination of collagenase gene expression revealed that the collagenase promoter/CAT construct activity was also enhanced by approximately three- to fivefold by LR. These observation are in a good agreement with those by Frisch et al. [1987], demonstrating coordinate regulation of stromelysin-1 and collagenase genes by phorbol esters and cytochalasin B. However, in our experiments, the collagenase mRNA steady-state levels were elevated up to about twenty- to thirtyfold [Mauviel et al., 1992; Table I], suggesting that additional mechanisms, such as stabilization of the collagenase mRNA, may potentially contribute to the differential elevation of collagenase mRNA levels. The transcriptional activation of stromelysin-1 gene expression by LR was shown to involve AP-1 *cis*-elements. Specifically, transfection of cells with the 1.3 kb promoter construct in which the AP-1 binding site was abolished by a single base substitution did not respond to LR in a similar manner as the parent construct containing the intact AP-1 sequence. Furthermore, our recent studies have demonstrated that LR induces *jun*-B gene expression [Mauviel et al., 1992], and the present results from gel mobility shift assays indicated that LR was inducing nuclear proteins that demonstrated a strong binding to a double-stranded oligonucleotide containing the AP-1 sequence. This binding was clearly specific and could be abolished by competition with a hundredfold excess of unlabeled AP-1 oligonucleotide. The autoradiograms of the gel shift assay demonstrated a predominant DNA-protein complex, but induction of three additional less abundant complexes by LR could also be detected. Thus, the protein present in the major protein-DNA complex is presumably the predominant regulator of gene expression. The significance of the three other bands representing protein-DNA complexes in relation to the major band is unclear at this point, but they may represent the induction of additional transcriptional factors in response to LR, which may interact with AP-1.

It was of interest to note that dexamethasone and all-*trans*-retinoic acid were able to counteract the LR-elicited up-regulation of stromelysin-1 gene expression. Recently, negative regulation of rat stromelysin gene promoter by retinoic acid has been shown to be mediated by an AP-1 binding site [Nicholson et al., 1990]. On the other hand, the inhibition of collagenase gene expression by dexamethasone has been shown to involve binding of the steroid receptor-ligand complex to the AP-1 regulatory proteins, thereby preventing their attachment to the corresponding *cis*-element [Yang-Yen et al., 1990]. Thus, these observations taken together provide strong evidence for the role of the transcription factor AP-1 in the regulation of stromelysin-1 gene expression by effector molecules. It should be noted, however, that the AP-1 sequence is not sufficient alone to mediate the LR-induced up-regulation of stromelysin-1 promoter activity, since no enhancement by LR in the expression of a construct (pAPCAT2a) containing three AP-1 sites in front of thymidine kinase promoter was observed. This observation is in good agreement with a recent demonstration that IL-1 and TPA induction of the stromelysin-1 promoter requires an additional neighboring up-

stream regulatory sequence which cooperates with AP-1 [Sirum-Connolly and Brinckerhoff, 1991]. It should also be noted that the basal level of expression of the stromelysin-1 promoter/CAT construct was considerably lower, only ~20% of the control level, when the AP-1 binding site was mutated by a single base substitution. Thus, in addition to the regulatory responses to various effector molecules, including LR, the AP-1 appears to play a role also in the basal expression of the stromelysin-1 gene.

In conclusion, LR, a T-cell derived cytokine, clearly up-regulates stromelysin-1 gene expression in human skin fibroblast cultures. It is conceivable, therefore, that in clinical situations characterized by inflammatory reactions which involve T-cells, stromelysin-1 activity may be increased as a result of LR induction of its gene expression. The elevated enzyme activity could lead to increased degradation of a variety of matrix molecules which have been shown to serve as substrates for proteolysis by stromelysin-1 [Woessner, 1991]. The degradation of the connective tissue components of the extracellular matrix is amplified further by activation of procollagenase by stromelysin-1 [Brinckerhoff et al., 1990]. Thus, it is conceivable that LR, in concert with other cytokines, such as TNF-α and IL-1, may initiate a cascade reaction leading to tissue destruction in inflammatory diseases.

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