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Competence for collagenase gene expression by tissue fibroblasts requires activation of an interleukin 1α autocrine loop

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ABSTRACT **The enzyme collagenase (EC 3.4.24.7), a key mediator in biological remodeling, can be induced in early-passage fibroblasts by a wide variety of agents and conditions. In contrast, at least some primary tissue fibroblasts are incompetent to synthesize collagenase in response to many of these stimulators. In this study, we investigate mechanisms controlling response to two of the conditions in question: (*i*) trypsin or cytochalasin B, which disrupt actin stress fibers, or (*ii*) phorbol 12-myristate 13-acetate (PMA), which activates growth factor signaling pathways. We demonstrate that collagenase expression stimulated by trypsin or cytochalasin B is regulated entirely through an autocrine cytokine, interleukin 1α (IL-1α). The IL-1α intermediate also constitutes the major mechanism by which PMA stimulates collagenase expression, although a second signaling pathway(s) contributes to a minor extent. Elevation of the IL-1α level in response to stimulators is found to be sustained by means of an autocrine feedback loop in early-passage fibroblast cultures. In contrast, fibroblasts freshly isolated from the tissue are incompetent to activate and sustain the IL-1α feedback loop, even though they synthesize collagenase in response to exogenous IL-1. We conclude that this is the reason why tissue fibroblasts are limited, in comparison with subcultured fibroblasts, in their capacity to synthesize collagenase. Activation of the IL-1α feedback loop, therefore, seems likely to be an important mechanism by which resident tissue cells adopt the remodeling phenotype.**

The collagenases (EC 3.4.24.7) appear to be the only mammalian enzymes with the capacity to catalyze cleavage of native soft-tissue collagens under physiological conditions (1), and they are thought to be important mediators of diverse tissue remodeling events, from wound healing to metamorphosis (2–5). Collagenolytic activity is regulated at multiple levels, including conversion of proenzyme to the activated form and complexing with specific inhibitors. However, collagenase is not constitutively synthesized by resident tissue cells, but is produced only when matrix degradation is demanded (6). Synthesis is, therefore, the primary level of regulation determining collagenolytic activity in tissues.

Investigations on collagenase synthesis have generally made use of early passage cultures of normal diploid fibroblasts. In these cells, collagenase synthesis is stimulated by a wide number of physiologically relevant agents. These include inflammatory cytokines, growth-promoting cytokines, and agents or conditions which disrupt the actin stress fibers that form as fibroblasts attach and spread on their substratum (2). Two nonphysiological agents, phorbol 12-myristate 13-acetate (PMA) and cytochalasin B (CB), mimic some of these conditions. PMA activates signaling pathways common to growth factors, due to its similarity to the second messenger, diacylglycerol (7). CB causes the depolymerization of actin filaments, thus reproducing physiologic conditions that disrupt actin stress fibers (8). An important finding that has emerged from studies with PMA or CB is that collagenase expression is not directly induced in response to these agents but is preceded by a lag time of 6–20 h (8, 9–11). In addition, the major increase in the level of collagenase mRNA is cotreatment of cells with cycloheximide (Cx), an inhibitor of protein synthesis (11). These findings demonstrate the requirement for an intermediate signal for induction of collagenase synthesis and further show that this signal is dependent on synthesis of an intermediate protein.

Recently, we identified the intermediate signal utilized by PMA as the inflammatory cytokine interleukin 1 (IL-1)—specifically, the IL-1α form (12). We did not investigate the nature of the intermediate through which CB stimulates collagenase synthesis; however, we suspect that IL-1α may be utilized in this case as well. This hypothesis is based on a secondary result of our study cited above, which revealed that constitutive collagenase synthesis is also regulated by the IL-1α autocrine. It has been suggested that the so-called "constitutive" synthesis might actually be due to the trypsin treatment used to remove cells from their plate for subculture, since this treatment disrupts actin stress fibers (13). Since IL-1α regulates "constitutive" collagenase synthesis, it then follows that other agents which disrupt actin stress fibers, such as CB, might also work through the IL-1α intermediate.

The mechanisms for regulating collagenase expression in early passage fibroblasts by CB or PMA have been studied and discussed at length. However, an important fact that has not been explained is this: cells freshly isolated from tissues, unlike early-passage subcultures, cannot respond properly to either CB or PMA (9, 14). This finding has led to the conclusion that at least some tissue fibroblasts are not competent to express collagenase, suggesting that acquisition of competence might, therefore, be an important control point in tissue remodeling. How could this competence be acquired? In view of our conclusions on the IL-1α intermediate with respect to PMA (12), and the hypothesis set forth above with respect to CB, it seems reasonable to propose that primary cultures might be unable to synthesize, or to respond to, IL-1α. In a previous publication from this laboratory, Girard *et al.* (15) showed that primary cultures treated with exogenous IL-1α produce normal amounts of collagenase. However, the hypothesis about incompetence to synthesize IL-1α remains to be tested.

In the following, we demonstrate the requirement for IL-1α in mediating collagenase gene expression in response to CB and PMA in primary corneal fibroblasts and passaged subcultures.

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Abbreviations: CB, cytochalasin B; Cx, cycloheximide; GAPD, glyceraldehyde-3-phosphate dehydrogenase; IL-1α, interleukin 1α; IL-1RA, interleukin 1 receptor antagonist; PMA, phorbol 12-myristate 13-acetate.

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MATERIALS AND METHODS

Fibroblast Culture and Treatment Reagents. Corneal stromal fibroblasts were isolated from rabbits and subcultured as previously described (9, 14). For studies on tissue fibroblasts (primary cultures), cells were plated for an experiment immediately after isolation. The following treatment reagents were utilized to stimulate collagenase synthesis: trypsin (Sigma) at 25 μg/ml (16), CB (Sigma) at 5 μg/ml (16), PMA (Sigma) at 1 μM (9, 12), and recombinant human IL-1 (R & D Systems) at 1 ng/ml (15). In some experiments, Cx (Sigma) was added at the same time as stimulators at 5 μg/ml. To interfere with IL-1 signaling, cells were treated with IL-1 receptor antagonist (IL-1RA; R & D Systems) or goat antiserum against rabbit recombinant IL-1α (Cytokine Sciences, Boston) at a range of doses. Nonimmune goat serum was used as a control for this latter reagent.

Analysis of Collagenase Protein Synthesis. Primary fibroblasts, or passaged fibroblasts freshly trypsinized from their culture dish, were plated at equal density (around 1.5×10^5 cells per well) in 24-well cluster dishes with medium containing 10% calf serum. The next day, this medium was replaced with 300 μl of serum-free medium and treatment reagents were added. Cells were treated with trypsin for 2–6 h, followed by addition of calf serum (final concentration: 10%) to inactivate the trypsin. [^{35}S]Methionine (New England Nuclear) was then added to culture medium at 80 μCi/ml (1 μCi = 37 kBq) for biosynthetic labeling of proteins. [^{35}S]Methionine was added at the same time as PMA or CB. Labeling was for 24 h. When required, IL-1RA or IL-1α antibody was added at the same time as [^{35}S]methionine. All treatments were performed in duplicate or triplicate.

After treatment, media were collected, and equal-sized samples were electrophoresed on SDS/8% polyacrylamide gels (9). Gels were autoradiographed and the total ^{35}S-labeled secreted protein in each gel lane was quantitated by volume densitometry (Molecular Dynamics). The relative amount of collagenase was determined by immunoprecipitation from pooled treatment replicates with 10 μl of sheep antiserum (17) or 25 μl of an "oligoclonal" mixture of five monoclonal antibodies reactive against rabbit collagenase 1 (18). In initial experiments, media were also analyzed with nonimmune sheep serum as a control for antibody specificity.

Quantitation of IL-1α Protein. Cells were treated with trypsin for 4 or 8 h, and treatment was terminated by addition of calf serum. CB treatment was performed for 4 or 24 h and PMA treatment for 24 h. Culture medium and cells were then collected from each well and assayed for total IL-1α content by radioimmunoassay (RIA) using a kit specific for the rabbit species (Cytokine Sciences) (19). Each sample was assayed in duplicate or triplicate, and determinations were averaged. The statistical significance of differences was determined by use of Student's *t* test. A value of $P < 0.05$ was considered significant.

RNA Analysis. For analysis of passaged cultures, confluent cultures were split 1:4 into 100-mm dishes and allowed to multiply until about 90% confluence. For analysis of primary cultures, fibroblasts freshly isolated from three corneas were plated in a 100-mm dish and used for an experiment the next day. Prior to starting an experiment, the culture medium was changed and treatment reagents were then added. After the appropriate treatment time (2–48 h), total RNA was isolated (20) and analyzed by Northern blotting. Rabbit cDNA probes for collagenase (21) and IL-1α (22) were labeled with ^{32}P by random priming (23). Loading equivalence between gel lanes was ascertained by probing for glyceraldehyde-3-phosphate dehydrogenase (GAPD) message with a human cDNA (24).

RESULTS

An Autocrine IL-1α Feedback Loop Acts as a Regulatory Intermediate in Response to Agents Which Disrupt Stress Fibers in Early-Passage Fibroblasts. We examined the capacity of trypsin and CB to stimulate expression of IL-1α and collagenase in early-passage fibroblast cultures (Fig. 1). Northern analysis (Fig. 1 *Left*) revealed a dramatic stimulation in collagenase mRNA levels 24 h after 2-h trypsin treatment; a smaller stimulation was observed with 6-h treatment. When this blot was reprobed with an IL-1α cDNA, it was found that trypsin, for both treatment times, also stimulated an increase in IL-1α mRNA. Importantly, the levels of IL-1α mRNA were increased coordinately with collagenase mRNA, with the higher level found in the cells treated for 2 h. In a related experiment, CB-treated cells were harvested at increasing times after treatment to determine the relative timing of the increase in collagenase and IL-1α mRNA. An increase in IL-1α mRNA could first be detected at 10 h after CB treatment; this clearly preceded the increase in collagenase message, which was not detectable until 15 h after treatment (Fig. 1 *Right*).

IL-1 expression can be regulated at the translational level (25), and, therefore, an increase in levels of the specific mRNA does not necessarily mean that an increase in protein levels will result. For this reason, RIA was used to make direct measurements of IL-1α protein concentrations in cell lysates after treatment with trypsin or CB. After only 4 h of trypsin treatment, the amount of IL-1α protein in cell lysates was significantly ($P = 0.002$) greater (7.6-fold) in treated (339.5 pg) as compared with untreated cultures (44.8 pg). With CB treatment, there was no difference between treated and untreated cells after 4 h. However, a significantly ($P = 0.03$) greater amount of IL-1α (1.9-fold) was found in cells treated with CB for 24 h (1665 pg) as compared with untreated cultures (865.5 pg). This timing for protein response to CB is consistent with the mRNA kinetics described above.

We further employed RIA to learn whether cytoskeletal rearrangement stimulates release of IL-1α into the cell culture medium. In this experiment, measurements were made only on CB-treated cells, since the proteolytic action of trypsin would preclude accumulation of intact IL-1α in the cell culture medium. After 4 h of CB treatment, the amount of IL-1α protein measured in the medium was not significantly different ($P = 0.5$) than in medium from untreated cells. However, after 24 h of treatment, a strikingly significant ($P = 0.006$) increase (7.7-fold) in the amount of IL-1α protein released into the medium was measurable as compared with controls.

To directly determine whether IL-1α acts as a mediator of collagenase synthesis stimulated by trypsin or CB, we treated cells with IL-1α antibody to neutralize cytokine activity. Trypsin treatment had no effect on the total protein synthesis.

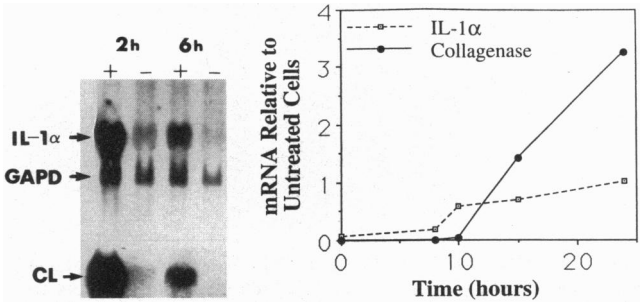

FIG. 1. Effects of trypsin and CB on IL-1α and collagenase mRNA levels. (*Left*) Cells were either treated with trypsin (+) for 2 or 6 h or left untreated (−), and RNA was collected 24 h later. This Northern blot was first probed for IL-1α and GAPD mRNAs, then it was stripped and reprobed for collagenase mRNA (CL). (*Right*) Paired cell samples treated with CB or left untreated were collected over 24 h. This blot was probed as in *A*, relative mRNA levels were quantitated by densitometry, and the untreated level was subtracted from the stimulated level for each time point graphed.

However synthesis of a protein electrophoresing with an apparent mass of 53 kDa, a size appropriate to be the proenzyme form of collagenase, was selectively stimulated (Fig. 2 *Upper*). Addition of the neutralizing antibody selectively blocked the synthesis of this protein, whereas normal goat serum had no effect. The identity of the stimulated 53-kDa protein was confirmed by immunoprecipitation with an antibody to rabbit collagenase (Fig. 2 *Lower*). Densitometric analysis revealed that collagenase synthesis was substantially reduced (2-fold) in trypsin-treated cells supplied with IL-1α antibody at 10 μg/ml. Synthesis was even further reduced (12.6-fold) in cells supplied with antibody at 100 μg/ml, to a level lower than in untreated cultures. In a matching experiment with CB, IL-1α antibody at 10 μg/ml reduced collagenase synthesis 3-fold and a 100-μg/ml dose reduced it 11.5-fold.

Naturally occurring IL-1RA can compete with IL-1α or IL-1β for binding to the IL-1 receptor, but it cannot transduce an intracellular signal (26, 27). To learn whether the effect of autocrine IL-1α was mediated through the IL-1 receptor, we treated cells with increasing doses of IL-1RA at the same time as CB. Similar to the results with IL-1α antibody, treatment with IL-1RA resulted in a dose-dependent reduction in CB-stimulated collagenase synthesis; at 100 ng/ml it was reduced by 2.6-fold and at 1000 ng/ml by 15.3-fold, ultimately to undetectable levels.

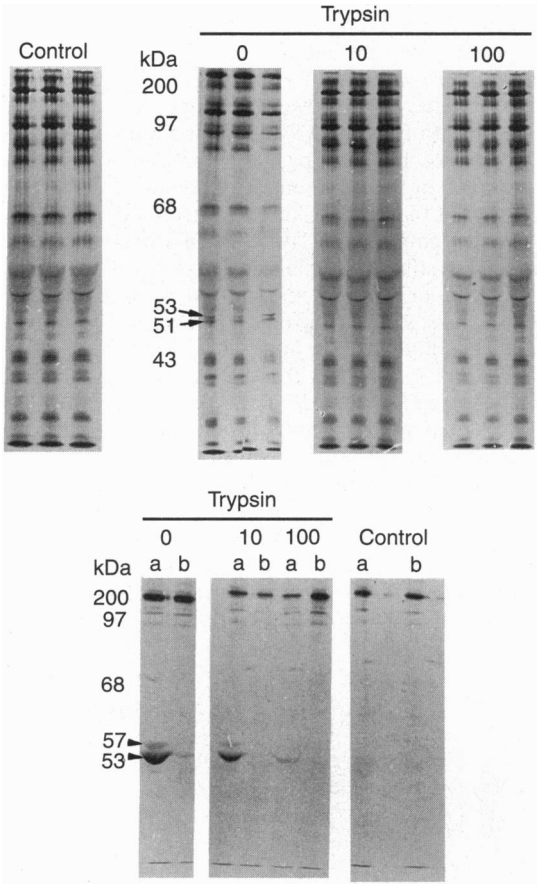

FIG. 2. Effects of IL-1α neutralization on trypsin-stimulated collagenase synthesis. (*Upper*) Culture medium was collected from triplicate wells of cells left untreated (Control) or treated with trypsin for 6 h along with IL-1α-neutralizing antibody at 0, 10, or 100 μg/ml. The autoradiograph displays the [^{35}S]methionine-labeled secreted protein profile. Arrows point to the 53- and 51-kDa proteins, corresponding to collagenase and the related metalloproteinase, stromelysin, respectively. (*Lower*) Replicate samples from each culture well analyzed in *A* were pooled and subjected to immunoprecipitation analysis with collagenase antiserum (lane a) or nonimmune serum (lane b). Arrowheads indicate the 53-kDa collagenase proenzyme and its 57-kDa glycosylated form.

It has been reported that IL-1α expression by fibroblasts can be controlled through a positive autoregulatory feedback mechanism (28). To learn whether positive feedback acts to sustain the levels of IL-1α stimulated by actin rearrangement, fibroblasts were treated with CB in the presence of IL-1RA at increasing doses, to antagonize IL-1α signal transduction through IL-1 receptors on the cell surface. Cell lysates and conditioned media were then analyzed by RIA. The results revealed a significant reduction in the amount of stimulated IL-1α in cell lysates from CB-treated cells supplied with IL-1RA (at 100 ng/ml reduced 9-fold and at 1000 ng/ml reduced 25-fold) as compared with cells treated with CB alone. The amount of IL-1α in the medium (released IL-1α) was also significantly reduced: at 100 ng/ml it was reduced 2.5-fold and at 1000 ng/ml it was reduced 6.8-fold.

Transition to the Collagenase-Producing Phenotype Requires Activation of the IL-1α Loop. To test our hypothesis that primary cells lack the capacity to synthesize IL-1α in response to CB and PMA, we assayed for IL-1α mRNA and protein levels in primary fibroblasts after treatment (Fig. 3*A*). Treatment with CB or PMA caused typical changes in cell shape in both primary and passaged cell cultures (not shown). In addition, both agents induced IL-1α mRNA (Fig. 3*A*) and collagenase mRNA (not shown) in passaged fibroblast cultures as expected from previous results. In contrast, CB did not induce IL-1α mRNA (Fig. 3*A*) or collagenase mRNA in primary fibroblasts (not shown). Furthermore, PMA induced IL-1α message only to low levels in primary cells. In fact, the result shown in Fig. 3 represents the highest level of IL-1α message induction that we have been able to achieve with PMA; in similar experiments, IL-1α mRNA has been lower or undetectable. Induction of collagenase mRNA did not occur to detectable levels in any of these experiments, although it was possible to detect induction of the protein product by the more sensitive technique of [^{35}S]methionine labeling (15). RIA analysis of IL-1α protein levels in cell lysates or conditioned

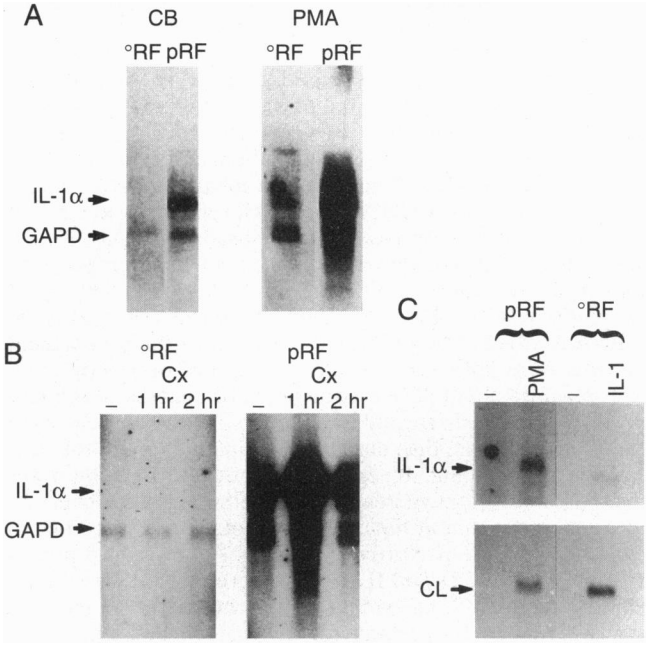

FIG. 3. Comparison of IL-1α expression in primary and passaged fibroblasts. Primary (°RF) or passaged (pRF) cultures were treated with CB or PMA for 24 h (*A*), treated with Cx for 1 or 2 h (*B*), or treated with PMA or exogenous IL-1α for 24 h (*C*). Northern blots of total RNA were hybridized with probes for IL-1α, collagenase (CL), or GAPD. –, Untreated controls.

media from primary cultures treated with CB or PMA paralleled our findings at the mRNA level.

Even when IL-1α message is undetectable in early passage fibroblast cultures, it can be induced to high levels by treatment with Cx (12, 28), a phenomenon which has been attributed to interference with specific mRNA turnover mechanisms. Fig. 3*B* depicts an experiment in which we compared the relative Cx inducibility of IL-1α mRNA in primary and passaged cultures. As previously reported (12), IL-1α mRNA levels were Cx inducible in passaged fibroblasts that were not expressing constitutive levels of the message. In contrast, neither 1 nor 2 h of Cx treatment induced detectable levels of IL-1α mRNA in primary fibroblasts.

To learn whether primary fibroblasts are capable of sustaining an IL-1α feedback loop, we examined the capacity of exogenously added IL-1 to induce IL-1α mRNA. Northern blot analysis revealed that treatment with exogenous IL-1 induced collagenase mRNA, comparable to the level expressed by a set of passaged cell cultures which were treated with PMA (Fig. 3*C*). In contrast, IL-1α mRNA was poorly induced in primary as compared with the passaged cultures.

Together, these results indicate that primary fibroblasts are capable of effective signaling through the IL-1 receptor, as evidenced by induction of collagenase mRNA. However, they cannot activate or sustain the IL-1α feedback loop.

Considering the results described above showing that PMA can weakly activate expression of IL-1α in primary cultures, we wondered whether the low collagenase induction by PMA (9) might be mediated by the IL-1α pathway. Primary fibroblasts were treated with PMA and simultaneously supplied with increasing doses of either IL-1α antibody or IL-1RA, to interfere with signal transduction mediated by IL-1α. In contrast to findings for passaged cultures, neither IL-1α antibody nor IL-1RA inhibited PMA-induced synthesis of the 53-kDa collagenase proenzyme in primary fibroblasts, even at the highest dose (not shown). These findings indicate that induction of collagenase synthesis by PMA in primary fibroblast cultures must operate through a regulatory pathway independent of the IL-1α intermediate.

DISCUSSION

In this report, we demonstrate that agents which disrupt actin stress fibers in early-passage fibroblasts activate an autocrine IL-1α feedback loop. This feedback loop then controls the subsequent stimulation of collagenase synthesis in a mechanism similar to that which we previously showed controls fibroblast response to PMA. Having established this, we then set out to determine whether the lack of autocrine IL-1α might explain the relative incompetence of primary tissue fibroblasts to synthesize collagenase in response to these stimulators. For these experiments, we made use of a corneal cell culture model described by Johnson-Muller and Gross in 1978 (14). These investigators observed that fibroblasts from the nonremodeling corneal stroma, when freshly isolated from the tissue, cannot elaborate collagenolytic activity in response to many agents that are stimulatory to the same cells after subculture. In a previous report, we reexamined this phenomenon more directly using molecular probes, and we found that primary cultures cannot synthesize collagenase constitutively or in response to CB. Furthermore, even the highest possible dose of PMA can stimulate only a small induction of collagenase—about 10% of the passaged cell response (9). These findings essentially confirmed the conclusion of the Gross group: that primary tissue fibroblasts are limited in their capacity to produce collagenase in response to CB or PMA.

The work of Kuter *et al.* (16) showed that, while CB treatment, alone, could not stimulate the elaboration of latent collagenolytic activity by primary cells, an increase in activity was stimulated when primary cultures were treated with exogenous IL-1 along with CB. Furthermore, conditioned medium from passaged corneal cell cultures could substitute for the purified cytokine. These results lead to the proposal that subculturing of cells stimulates the synthesis of a biologically active cytokine which could act as a cofactor for CB. In fact, when we assayed collagenase synthesis directly, we found that CB was not essential for stimulation; primary cultures treated only with IL-1 synthesize levels of collagenase similar to passaged cultures (15). Nevertheless, in view of conclusions from our earlier work about the role of IL-1α in mediating collagenase synthesis in passaged cultures (12), we were still led to a hypothesis similar (although more specific) to that of the Gross group: the inability of primary cells to respond properly to CB or PMA is due to their incompetence to synthesize the IL-1α intermediate.

Our hypothesis turned out to be essentially correct. As predicted, it was found that primary cultures cannot synthesize IL-1α in response to CB. On the other hand, PMA could stimulate a small induction of IL-1α synthesis. However, the low level of collagenase synthesis stimulated by PMA appears to be controlled by a mechanism independent of IL-1α, since cotreatment with IL-1α antagonists failed to inhibit collagenase synthesis. Why does CB or PMA fail to induce IL-1α to any substantial level in primary cultures? Our experiments with Cx suggested that the IL-1α gene is being actively transcribed in passaged cells but that the mRNA has a very short half-life and does not accumulate unless cells are treated with a stimulator. In contrast, a similar experiment with primary cultures suggested that the IL-1α gene is not being transcribed. Furthermore, the IL-1α mRNA levels are not stimulated by exogenously added IL-1 in primary cultures. Together, these results indicate that primary cultures are deficient in their capacity to activate or to sustain the IL-1α feedback loop. Surprisingly, although the human IL-1α gene was characterized some years ago (22), essentially nothing about the mechanisms regulating its transcription has been reported in the literature. Such studies will be paramount if the impaired responsiveness to stimulators in primary cultures is to be understood.

Although we utilized corneal fibroblasts in this study, we have previously demonstrated that subcultured fibroblasts derived from other tissues also utilize the IL-1α intermediate to control collagenase synthesis (12). Unfortunately, preparation of a pure population of fibroblasts directly from tissues is generally not possible because of the heterogeneity of cell types that they contain. For this reason, the transition to collagenase competence has not been documented in other tissue types besides cornea, which has a uniquely homogeneous cell composition (29). Nevertheless, it seems likely that fibroblasts freshly isolated from other nonremodeling tissues would behave similarly to those from cornea. In fact, it was recently reported that IL-1 is the intermediate controlling collagenase synthesis by rat myometrial cells in response to serotonin (30). Interestingly, these cells, like corneal stromal cells, must also undergo a developmental transition in culture before they are competent to synthesize IL-1 and collagenase in response to serotonin. This finding reveals that not only is the IL-1 mechanism operative in a variety of mesenchymal cell types but also its importance crosses species.

It has long been known that IL-1 stimulates collagenase expression during inflammatory processes, and there are many biological examples of this regulation (26, 31, 32). However, in the inflammatory context, IL-1 acts as a paracrine, mediating tissue interaction between inflammatory cells and resident tissue fibroblasts. There are no examples, to our knowledge, that mechanistically demonstrate that the autocrine mechanism is utilized in biological remodeling. However, we are aware of a few examples in which IL-1α and collagenase have been colocalized in the same cells. For example, it is reported that collagenase and IL-1α protein are both expressed by mesenchymal cells during the acute remodeling phase of heart

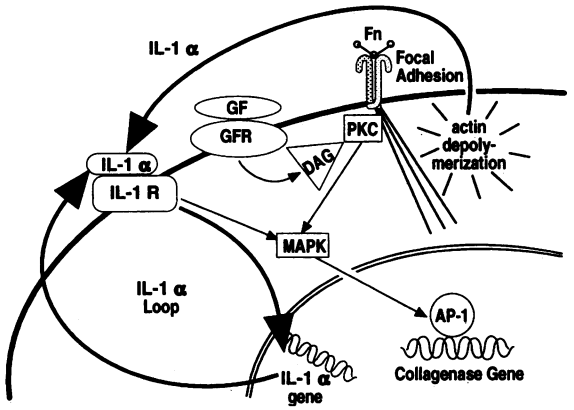

FIG. 4. Proposed feedback loop model for the regulation of collagenase gene expression by the IL-1α autocrine. DAG, diacylglycerol; Fn, fibronectin; GF, growth factor; GFR, growth factor receptor; IL-1 R, IL-1 receptor; MAPK, mitogen-activated protein kinase; PKC, protein kinase C.

morphogenesis in the rat (33). Expression of IL-1α has also been colocalized with collagenase in metastatic cancer cells (34). Activating the autocrine IL-1α loop, therefore, seems likely to be an essential mechanism by which fibroblasts make the transition to the remodeling phenotype *in vivo*.

The current results, viewed in combination with findings from our previous studies (9, 12, 15), lead us to propose a model for regulation of collagenase gene expression in passaged fibroblasts (Fig. 4). We have depicted actin stress fibers assembled on the integrin receptor for fibronectin, the matrix attachment factor found in the serum used for our cell plating (18). Upon their disruption, release of IL-1α might result from the exocytosis of intracellular vesicles within which IL-1α is known to accumulate in both monocytes and fibroblasts (26, 34). Once outside the cell, IL-1α is free to bind IL-1 receptors on the surface of the releasing cell or on the surface of a neighboring cell. Receptor ligation would activate the IL-1α feedback loop, stimulating new IL-1α synthesis via a pathway which remains to be characterized. Ligation of the IL-1 receptor by IL-1α would also activate transcription of the collagenase gene. The mechanism whereby this occurs is poorly defined; however, recent evidence implicates a pathway involving a mitogen-activated protein kinase (MAPK). This ultimately activates AP1 (35), a transcription factor composed of members of the c-*fos* and c-*jun* oncogene families that is essential for collagenase gene transcription (36). PMA binds to, and activates, protein kinase C (PKC) by virtue of its similarity to the second messenger released as a result of ligation of growth factor receptors (7). This also sets off a MAPK–AP1 signaling pathway (37). However, PMA is also known to cause actin cytoskeletal rearrangements (38). We propose that this effect is the pathway by which PMA activates the IL-1α feedback loop. Alternatively, however, PMA might also activate the IL-1α loop through a direct signaling pathway that is as yet undefined.

The concept of regulation through the IL-1α autocrine intermediate constitutes a useful paradigm for understanding collagenase gene expression. It was realized some years ago that regulation of collagenase expression in response to stimulators occurs primarily by indirect mechanisms (17). However, this has been largely forgotten in the excitement over new findings on gene activation via the AP1 signaling pathway, a mechanism thought to be independent of new protein synthesis (36, 37). Nevertheless, while our findings point to the IL-1α intermediate as the major pathway to the collagenase gene in cultured cells, they also make it clear that alternative pathways do exist. Further studies will be required to identify the relative importance of alternative pathways controlling collagenase expression in situations *in vivo*.

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