Pergamon

PII: S1357-2725(97)00030-7

Int. J. Biochem. Cell Biol. Vol. 29, No. 6, pp. 901–910, 1997

Printed in Great Britain
1357-2725/97 $17.00 + 0.00

Lung Alveolar Epithelial Cells Synthesize Interstitial Collagenase and Gelatinases A and B *In Vitro*

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Type II pneumocytes are multifunctional alveolar epithelial cells that play a major role in the maintenance of lung structure and function. Recent evidence supports that these cells can synthesize a variety of extracellular matrix components *in vitro*, suggesting an active participation in connective tissue remodeling. However, their possible role in extracellular matrix degradation is unknown. In this study the production of matrix metalloproteinases (MMPs) was examined in primary cultures of rat alveolar type II pneumocytes after 2 and 7 days in culture. Under basal conditions, at both periods type II cells expressed interstitial collagenase mRNA. The immunoreactive protein was detected both in the cells and in conditioned media, and collagenolytic activity was revealed after trypsin activation. Gelatinolytic activity was detected by zymography showing a relative molecular mass of ~72 and 92 kDa (gelatinases A and B). Phorbol treatment increased collagenase and gelatinase activities. In addition, three alveolar epithelial cell lines were analysed for MMP production: MLE-12 (mice), L2 (rat), and A549 (human). The cell lines A549 and MLE-12 revealed collagenase and gelatinase A and B activities whereas the L2 cell line only exhibited gelatinase A activity, even after PMA induction. These findings demonstrate that alveolar epithelial cells synthesize *in vitro* several MMPs that confer on them the ability to degrade extracellular matrix and basement membrane components, a capacity of considerable importance for the remodeling of the stromal/epithelial interface.

Keywords: Type II pneumocytes Collagenase Gelatinase Extracellular matrix

Int. J. Biochem. Cell Biol. (1997) **29**, 901–910

INTRODUCTION

Alveolar type II epithelial cells play a major role in the maintenance of lung structure and function (Castranova *et al.*, 1992). These cells are the progenitors of type I pneumocytes and are the primary source of pulmonary surfactant. In addition, alveolar type II cells can synthesize a variety of extracellular matrix components *in vitro*, including fibronectin, type IV collagen, laminin and proteoglycans, suggesting that they actively participate in basement membrane and connective tissue metabolism (Dunsmore and Rannels, 1995; Rannels *et al.*, 1987; Simon *et al.*, 1993). However, their possible role in the degradative pathway of interstitial matrix and basement membrane remodeling has not been demonstrated.

Matrix metalloproteinases (MMPs) constitute a family of structurally related matrix-degrading enzymes, which play a key role in the physiologic turnover of connective tissue as well as in the pathological remodeling observed in several disorders (Pardo and Selman, 1991; Matrisian, 1992).

The MMP family, a group of Zn^{2+} proteinases, consists of different subclasses including collagenases, gelatinases, stromelysins and membrane-type metalloproteinases (Matrisian, 1992; Murphy *et al.*, 1992; Sato *et al.*, 1994).

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Received 27 August 1996; accepted 14 February 1997.

Collagenases cleave the triple helical region of interstitial collagen types I, II and III, generating 3/4 and 1/4 collagen fragments (Welgus *et al.*, 1981). Three highly homologous human collagenases, fibroblast (MMP-1), neutrophil (MMP-8) and collagenase-3 (MMP-13) have been cloned (Goldberg *et al.*, 1986; Hasty *et al.*, 1990; Freije *et al.*, 1994). Rat and mice collagenases share the highest degree of homology with human collagenase-3 (Henriet *et al.*, 1992; Quinn *et al.*, 1990), there being no evidence to date for a homologous MMP-1 in either rat or mouse. The gelatinase subclass is composed of two members, 72 and 92 kDa type IV collagenases (MMP-2/gelatinase A and MMP-9/gelatinase B, respectively). They have the ability to degrade type IV collagen, the major structural component of basement membranes, type V collagen, denatured collagens (gelatin) of all genetic types and insoluble elastin (Collier *et al.*, 1988; Wilhem *et al.*, 1989; Senior *et al.*, 1991). The stromelysins subclass has broad substrate specificity including proteoglycans, fibronectin and laminin (Murphy and Docherty, 1992).

Interstitial collagenases are primarily produced by connective tissue cells and inflammatory cells. The 72 kDa type IV collagenase is constitutively expressed by most fibroblastic cells, while the 92 kDa gelatinase is secreted by monocytes/macrophages and polymorphonuclear leukocytes, as well as by several transformed cell lines (Murphy and Docherty, 1992). In addition, a growing body of evidence strongly suggests that keratinocytes, as well as other cells of epithelial lineage, are able to express several members of the matrix metalloproteinase gene family either constitutively or after stimulation (Lin *et al.*, 1987; Salo *et al.*, 1991; Rolland *et al.*, 1995). However, the possible production of metalloproteinases by adult lung alveolar epithelial cells has not been previously reported.

We have hypothesized that type II pneumocytes might participate in the matrix and basement membrane remodeling not only through the production of matrix components but also by secreting several metalloproteinases. To test this hypothesis we examined *in vitro* the capacity of alveolar type II cells derived from adult rat lungs, to secrete interstitial collagenase and gelatinases. Additionally three alveolar epithelial cell lines, specifically MLE-12 (mice), A549 (human) and L2 (rat), were also tested for their ability to secrete these metalloproteinases.

MATERIALS AND METHODS

Alveolar epithelial cell isolation and culture

Primary cultures of rat alveolar type II pneumocytes (AT2) and three lung epithelial cell lines: MLE-12 (mice), L2 (rat) and A549 (human) were used (Douglas and Kaighn, 1974; Smith, 1977; Wikenheiser *et al.*, 1993). MLE-12 cells were a generous gift from J. Whitsett, and L2 and A549 were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.)

Type II pneumocytes were isolated as previously described (Olivera *et al.*, 1994). Briefly, the lungs were perfused via the pulmonary artery, lavaged and digested with elastase, 30 U/ml (Worthington Biochemical, Freehold, NJ, U.S.A.) for 20 min at 37°C. The tissue was minced and filtered through sterile gauze and 70 μm nylon mesh. The cell population was enriched for type II pneumocytes by panning the cellular suspension over a surface coated with rat IgG to remove cells with Fc receptors. Viability and purity of the final cell preparation exceeded 90%.

AT2 cells were cultured in Dulbecco's minimal essential medium (DMEM) containing 2 mM glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin and 10% fetal calf serum. Cells were seeded on tissue culture dishes at 2.5×10^5 cells/cm^2 and incubated at 37°C in 5% CO_2, 95% air.

After 1 and 6 days of culture, cells were changed to serum-free medium for 24 hr, and the assays actually represent 2 and 7 days post-isolation. Parallel experiments were performed in the presence of 60 ng/ml of phorbol-12-myristate-13-acetate (PMA).

Under the culture conditions employed, the number of alveolar epithelial cells did not change from day 1 to 7, as has been previously demonstrated elsewhere (Uhal *et al.*, 1991). Likewise, PMA did not exhibit a cytotoxic effect or modify the cell number of primary alveolar epithelial cells.

L2, MLE-12 and A549 cell lines were grown in the same conditions until early confluence and were then changed to serum-free medium in the absence or presence of 60 ng/ml of PMA for 24 hr. Conditioned media (CM) were collected and stored at −20°C until assayed.

Additionally, rat lung fibroblast cell lines were obtained in our laboratory as previously described (Medina *et al.*, 1994). Cells were cultured under standard conditions and at early

confluence cells were changed as above to serum-free medium in the absence or presence of PMA for 24 hr. Fibroblasts were collected for RNA extraction.

Assay for collagenolytic activity

Collagenase activity was measured as described elsewhere (Pardo *et al.*, 1991) by the method of Terato *et al.* (Terato *et al.*, 1976) using as substrate native guinea pig skin type I collagen labeled with [3H]-acetic anhydride. For each assay 100 μl of a 1.7 mg/ml collagen solution with a specific activity of 1.3×10^6 cpm/mg were used. The maximal sensitivity of the collagen to degradation by trypsin was always less than 7% at a ratio of 1:100 (trypsin/collagen), and collagenase activity was calculated after subtraction of trypsin blanks. Serum-free CM were concentrated 10-fold using Centricon 10 concentrators (Amicon, Beverly, MA, U.S.A.). To activate latent collagenase, samples were preincubated with trypsin (1–5 μg) for 10 min at 37°C followed by addition of 5-fold excess of soybean trypsin inhibitor. Activated samples were incubated with labeled collagen for 18 hr at 30°C. Collagenolytic activity was calculated after background subtraction and the results of the enzyme activity are expressed as μg of collagen degraded at 30°C/18 hr.

Zymography

CM (10-fold concentrated) derived from serum-free or PMA stimulated cells (20 μl) were incubated at room temperature for 30 min with Laemmli sample buffer (20 μl) containing 3% SDS. Aliquots (15 μl) were resolved under non-reducing conditions in 7.5% gel copolymerized with 1 mg/ml gelatin or casein (Sigma, St Louis, MO, U.S.A.) (Heusen and Dowdle, 1980). To activate progelatinases, some samples were preincubated with 1 mM *p*-aminophenylmercuric acetate (APMA) (Sigma Chemical Co., St Louis, MO, U.S.A.) for 2 hr at 37°C. After electrophoresis, the gel was washed at room temperature twice for 15 min with 2.5% Triton X-100 followed by several washes with distilled water and was then incubated overnight at 37°C in 50 mM Tris–HCl buffer, pH 7.5, 5 mM $CaCl_2$, 20 μM $ZnCl_2$. The gel was stained with Coomassie brilliant blue R-250. Molecular weights of the gelatinolytic or caseinolytic bands were estimated by using prestained molecular weight markers (BioRad, Hercules, CA, U.S.A.). To analyse metalloproteinase inhibition, gels were incubated in the presence of 20 mM EDTA.

Immunocytochemistry

Freshly isolated type II pneumocytes were cultured for 2 days on coverslips as mentioned above, and then washed with PBS and fixed with carbowax. Endogenous peroxidase was blocked by incubation with 0.3% H_2O_2 in methanol for 5 min. Cell membranes were permeabilized with 0.1% Triton X-100 and cells were blocked with non-immune rabbit IgG (diluted 1/20 in PBS) for 20 min. Cells were then incubated with anti-rat interstitial collagenase rabbit IgG (50 μg/ml), kindly provided by J. Windsor, University of Alabama at Birmingham (Lin *et al.*, 1987). After 4 hr incubation at room temperature, the cells were washed with PBS and treated with a secondary biotinylated anti-rabbit antibody (Vector Labs Inc., Burlingame, CA, U.S.A.), diluted 1:150. Avidin–biotin peroxidase complex was applied for 30 min followed by 3,3′-diaminobenzidine in PBS containing 0.045% H_2O_2. The primary antibody was replaced by normal rabbit serum for negative control slides.

Immunoblot analysis

Serum-free CM were centrifuged at 300 ***g*** at 4°C for 30 min to remove cell debris and concentrated by lyophilization. Samples were solubilized in 500 μl of distilled water and 10 μl were mixed with the same volume of Laemmli sample buffer and electrophoresed on 10% SDS–PAGE (Laemmli, 1970). Western transfer to a nitrocellulose filter was performed at 70 V for 3 hr. After the non-specific sites were blocked overnight with 4% (w/v) non-fat dried milk in PBS, the membrane was incubated with anti-rat interstitial collagenase rabbit IgG for 2 hr. Reaction with secondary antibody conjugated to peroxidase was for 1 hr at room temperature. Finally, the filter was incubated for 15–20 min at room temperature in PBS containing 0.15% H_2O_2, 15% (vol/vol) methanol, and 4-chloronaphthol at 6 mg/ml until the color developed.

RNA isolation and Northern blot

Total cellular RNA from rat alveolar epithelial cells and fibroblasts was isolated by the acid guanidinium thiocyanate/phenol chloroform extraction method (Chomczynski *et al.*, 1987). Total RNA (15 μg/lane) was fractionated on a 1% agarose–formaldehyde gel. RNA

Fig. 1. Northern blot analysis for rat interstitial collagenase. Equal amounts of total RNA (15 μg/lane) isolated from rat fibroblasts (lane A), and alveolar type II epithelial cells after 2 and 7 days of culture (lanes B and C) were electrophoresed on agarose gel, blotted and hybridized with ^{32}P cDNA probe. The membranes were exposed for 4 days at −70 C.

integrity was assessed by observing the appearance of the ribosomal RNAs. The fractionated RNA was transferred onto Nytran membrane and then fixed by baking at 80°C for 2 hr. The cDNA clone for rat collagenase was kindly provided by C. O. Quinn (St Louis University, St Louis, MO, U.S.A.) (Quinn *et al.*, 1990). The membranes were hybridized and washed as described elsewhere (Medina *et al.*, 1994).

RESULTS

Primary cultures of type II pneumocytes: interstitial collagenase

The expression and synthesis of rat interstitial collagenase by type II pneumocytes *in vitro* was investigated after 2 and 7 days in culture [AT2(2) and AT2(7)]. It has been suggested that after 7 days of culture type II cells achieve some morphologic and metabolic characteristics of

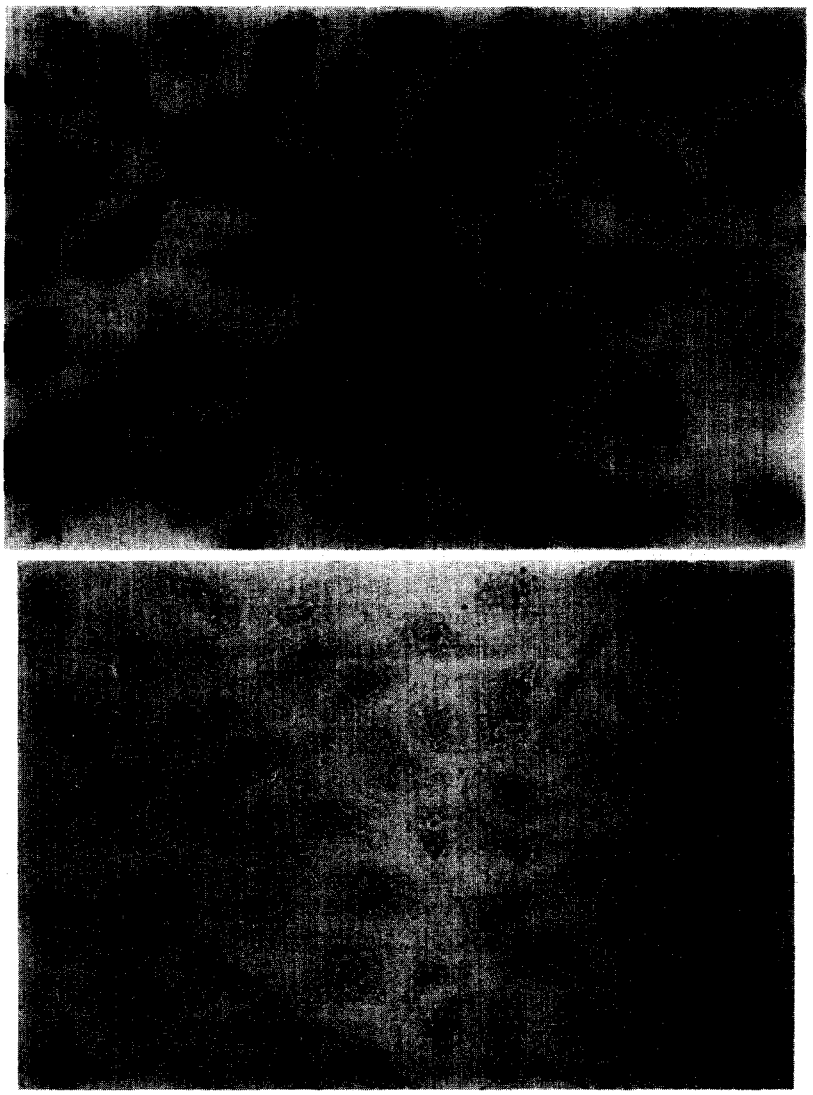

Fig. 2. Immunocytochemistry for interstitial collagenase in type II pneumocytes. (A) Primary cultures of 2-day-old rat alveolar epithelial cells stained for MMP-13 with anti-interstitial collagenase rabbit IgG showing perinuclear cytoplasmic staining. (B) Cells without positive cytoplasmic staining when primary antibody was replaced with non-immune rabbit IgG.

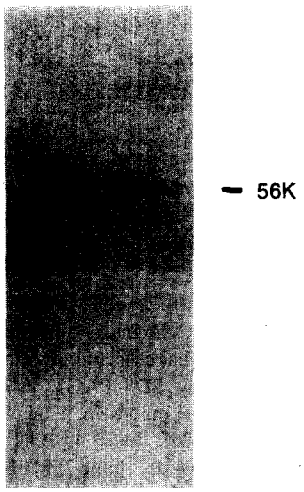

Fig. 3. Western blot analysis of interstitial collagenase in conditioned media obtained from type II pneumocytes. Conditioned media derived from primary cultures of rat AT2 after 2 days (lane 1) and 7 days (lane 2) of culture were collected, electrophoresed on 10% SDS–polyacrylamide gel electrophoresis gels, and transferred to nitrocellulose. Immunoblotting was performed with anti-interstitial collagenase rabbit IgG. Relative molecular weight is indicated.

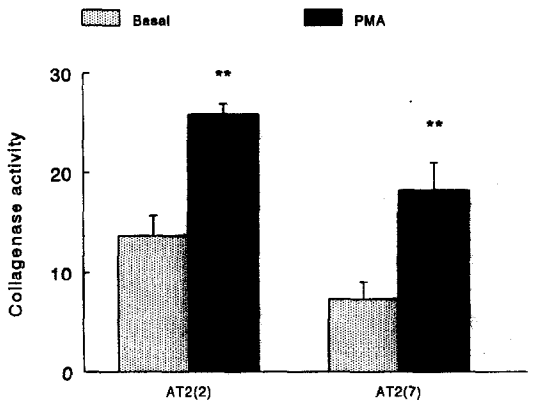

Fig. 4. Collagenolytic activity in serum-free (solid bars) and PMA conditioned media (hatched bars) derived from primary cultures of type II pneumocytes [AT2(2) and AT2(7)]. CM derived from $\sim 5.2 \times 10^6$ cells were 10-fold concentrated and activated by trypsin partial digestion as described in Materials and Methods. Collagenase activity is expressed as μg of type I collagen degraded per 18 hr at 30°C. Values shown represent mean ± SD for three different cell preparations. $^{**}P < 0.01$.

type I epithelial cells (Rannels and Rannels, 1989).

Northern blot analysis showed that alveolar epithelial cells express a ~3.0 kb collagenase transcript both after 2 and 7 days of culture (Fig. 1, lanes B and C). This transcript was similar to the mRNA size from stimulated rat fibroblasts used as positive control (Fig. 1, lane A).

The presence of immunoreactive protein was observed by immunocytochemistry in some clusters of cultured alveolar epithelial cells exhibiting typical morphology with the presence of lamelar bodies. A clear cytoplasmic immunolabeling was detected when these cells were treated with specific antibody against interstitial collagenase (Fig. 2A). Cells incubated with pre-immune IgG did not reveal any staining (Fig. 2B).

Immunoreactive collagenase was also detected as a ~56 kDa protein band by Western blot analysis in serum-free CM derived from AT2(2) and AT2(7) cells (Fig. 3).

For collagenolytic activity assay, cell culture dishes of 21 cm^2 of growth area containing $\sim 5.2 \times 10^6$ type II cells were used; also 5 ml of serum-free and PMA-CM derived from AT2(2) and AT2(7) primary cultures were 10-fold concentrated and activated by trypsin partial digestion. Results derived from three different

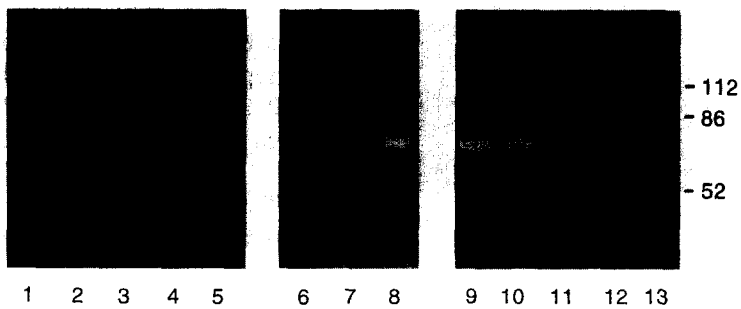

Fig. 5. Zymogram with gelatin as substrate. Conditioned media derived from primary cultures of type II pneumocytes AT2(7) and AT2(2) were resolved by SDS–PAGE using a 7.5% gel copolymerized with 1 mg/ml gelatin. AT2(7) under basal conditions (lanes 4 and 5), preincubated with 1 mM APMA (lane 2), and after PMA (60 ng/ml) stimulation for 24 hr (lane 3). Conditioned media from AT2(2) cells under basal conditions (lanes 8, 11 and 12), after preincubation with APMA (lane 7), and after PMA stimulation (lanes 9 and 10). Lanes 1, 6 and 13 represent prestained molecular weight markers. Zones of enzymatic activity appear as clear bands against a dark background. Gelatinolytic activity was inhibited by EDTA.

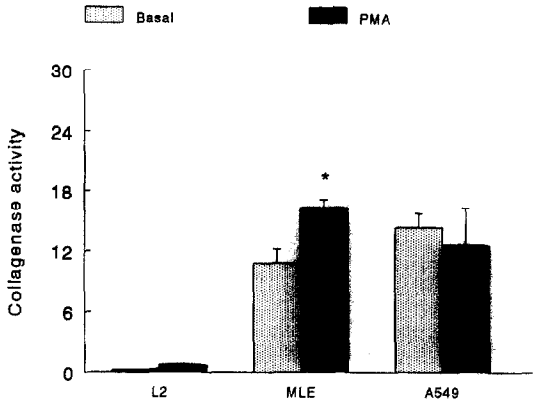

Fig. 6. Collagenolytic activity assayed in serum-free and PMA-CM derived from L2, MLE and A549 cell lines. A 10 ml sample derived from 5.44×10^6 (L2), 8.06×10^6 (MLE) and 3.08×10^6 (A549) were 10-fold concentrated and activated by trypsin partial digestion. Collagenase activity is expressed as μg of type I collagen degraded per 18 hr at 30°C. Values shown represent mean $\pm$ SD for three different cell preparations. $^*P < 0.05$.

cell preparations are shown in Fig. 4. Under basal conditions, type II cells revealed collagenolytic activity, which was significantly increased by PMA stimulation ($P < 0.01$).

Type IV collagenases/gelatinases

Zymography with gelatin substrate was used to monitor the presence of gelatinases in serum-free CM from unstimulated and PMA-stimulated cells. Gelatin zymography of non-reduced CM revealed a constant major ~72 kDa proteolytic band, the activated latent form of gelatinase A, both in AT2(7) (Fig. 5, lanes 4 and 5) and AT2(2) (Fig. 5, lanes 8, 11 and 12) primary cultures. These gelatinolytic

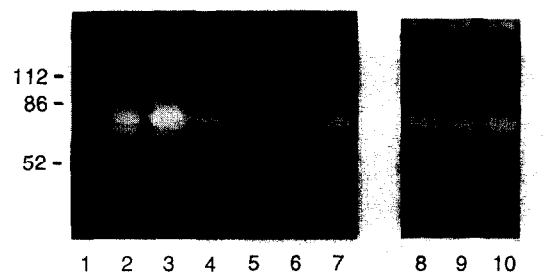

Fig. 7. Gelatin zymogram. Conditioned media derived from: L2 cell line under basal conditions (lane 3), preincubated with 1 mM APMA (lane 2) and after PMA (60 ng/ml) stimulation for 24 hr (lane 7); MLE cell line under basal conditions (lane 6), with APMA (lane 5) and with PMA stimulation (lane 4); A549 cell line under basal conditions (lane 8), preincubated with APMA (lane 9) and after PMA (lane 10). CM were resolved by SDS–PAGE using a 7.5% gel copolymerized with 1 mg/ml gelatin. Lane 1: prestained molecular weight markers.

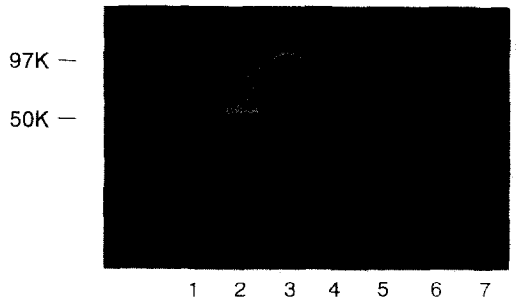

Fig. 8. Zymogram with casein as substrate. Conditioned media of alveolar epithelial cells and alveolar macrophages (AM) were resolved by SDS-PAGE using a 10% gel copolymerized with 1 mg/ml casein. Lane 1: AM. Lane 2: A549. Lane 3: AT2 (2d). Lane 4: AT2 (7d). Lane 5: MLE-12 + PMA. Lane 6: L2. Lane 7: MLE-12. Prestained molecular weight markers are shown to the left. Numbers indicate relative molecular weight.

bands were also observed when the CM were diluted 1:3 (Fig. 5, lanes 4 and 11), and 1:10 (Fig. 5, lane 12). When the conditioned media were treated with APMA the MMP-2/gelatinase A activity was partially converted to a 68 kDa and a minor 62 kDa active form in AT2(7) and to a 62 kDa active form in AT2(2) cells (Fig. 5, lanes 2 and 7, respectively). After PMA stimulation, the 68 kDa active form was increased, suggesting active conversion of the latent into active form as can be observed in Fig. 5, lane 3, for AT2(7) and lanes 9 and 10 for AT2(2).

Primary cultures of type II pneumocytes constitutively expressed low levels of a ~92 kDa gelatinase activity that is the appropriate size to be gelatinase B proenzyme. After PMA stimulation an increase in the 92 kDa gelatinolytic band was observed in both AT2(7)- and AT2(2)-derived CM (Fig. 5, lanes 3, 9 and 10). Organomercurial activation of CM with APMA resulted in the conversion of the 92 kDa gelatinase into a species of about 88 kDa that would represent the active MMP-9 form (Fig. 5, lanes 2 and 7). All gelatinolytic bands were inhibited by 20 mM EDTA (data not shown).

Alveolar epithelial cell lines

Collagenolytic activity was assayed in serum-free and PMA-CM derived from L2, MLE and A549 cells grown until early confluence in T-75 flasks. Ten ml derived from 5.44×10^6 (L2), 8.06×10^6 (MLE) and 3.08×10^6 (A549) were 10-fold concentrated and activated by trypsin partial digestion. Results derived from three cell culture flasks are shown in Fig. 6. The CM from L2 cell line did not reveal collagenolytic activity

under the present experimental conditions, even after PMA stimulation. The MLE-12 cell line showed a similar behavior to primary type II cells. Collagenolytic activity was present under basal conditions and it was significantly increased by PMA stimulation ($P < 0.05$). A549 cell line also exhibited the ability to degrade collagen under basal conditions, although this activity was not enhanced when cells were previously stimulated by PMA.

Gelatin zymography of non-reduced CM revealed in L2 cell line, a 72 kDa activated latent form together with a 68 and a 62 kDa active form (Fig. 7, lane 3). APMA activation increased active forms (Fig. 7, lane 2) whereas PMA stimulation did not show any effect on gelatinase A activities (Fig. 7, lane 7). L2 cell line did not reveal gelatinase B activity either under basal conditions or after PMA exposure. This CM displayed a higher molecular weight band (~140 kDa) that might correspond to gelatinase A dimers. Gelatinase activities were fully inhibited by 20 mM EDTA (data not shown).

MLE cell line exhibited a MMP-2/gelatinase activity of 72 kDa, as well as a 68 kDa and a minor 62 kDa active form (Fig. 7, lane 6). When CM were activated with APMA, the 68 and 62 kDa active forms increased (Fig. 7, lane 5). With PMA stimulation, a new band of ~92 kDa representing gelatinase B activity was observed (Fig. 7, lane 4). EDTA inhibited all gelatinolytic bands (not shown).

A549 cell line showed a large number of gelatinolytic bands, including a constant major ~72 kDa proteolytic band (Fig. 7, lane 8) which was partially converted to the 62 kDa active form with APMA treatment (Fig. 7, lane 9). A 92 kDa band (gelatinase B) was also observed under basal conditions and that species was converted to a 88 kDa form after APMA treatment (Fig. 7, lanes 8 and 9). PMA stimulation did not show any effect on gelatinase A, but a slight increase in gelatinase B intensity (Fig. 7, lane 10). A549 cell line exhibited in addition, a 30 kDa gelatinolytic band. With the exception of some high molecular weight gelatinolytic bands (~200 kDa) gelatinase activities were inhibited by EDTA (not shown).

Casein zymography

Casein zymography (Fig. 8) revealed a ~92 kDa lytic activity in CM derived from type II cells after 2 and 7 days in culture (Fig. 8, lanes 3 and 4), as well as in the A549 and PMA-stimulated MLE-12 cell lines (Fig. 8, lanes 2 and 5). CM derived from rat alveolar macrophages used as control, displayed enzymatic activities at 92 kDa and 86 kDa (Fig. 8, lane 1). On the other hand the L2 cell line that did not reveal gelatinase B activity on gelatin gels but presented a strong gelatinase A (MMP-2) activity, showed no caseinolytic activity (Fig. 8, lane 6). The MLE-12 and A549 cell lines exhibited an additional caseinolytic activity of ~55 kDa that might correspond to stromelysin. Additionally, a 30 kDa band was observed in alveolar macrophage CM. All these caseinolytic bands were inhibited by 20 mM EDTA (data not shown). A549 also showed on casein gels high molecular weight bands that were not inhibited by EDTA.

DISCUSSION

The role of alveolar epithelial cells in the remodeling of the stromal/epithelial interface has been studied primarily with respect to the synthesis of basement membrane components. Several reports show that at least *in vitro*, type II pneumocytes are able to produce fibronectin, type IV collagen, laminin and proteoglycans (Dunsmore and Rannels, 1995; Rannels *et al.*, 1987; Simon *et al.*, 1993).

We reasoned that these multifunctional cells could also play a role in the degradation of basement membrane and extracellular matrix components. Our findings demonstrate that rat type II alveolar epithelial cells *in vitro* produce interstitial collagenase (MMP-13), gelatinase A (MMP-2) and gelatinase B (MMP-9). Additionally, we found that stimulation with PMA caused an increase in collagenolytic activity as well as gelatinase B activity. This effect has been well documented for interstitial collagenase in a variety of mesenchymal cells and for 92 kDa gelatinase B in alveolar macrophages, osteoclasts and keratinocytes (Matrisian, 1992; Murphy and Docherty, 1992; Lyons *et al.*, 1993).

Type II cells have garnered attention because, in addition to a broad range of activities, they serve as progenitor cells for the alveolar epithelium (type I cells). It has been also suggested that after several days in culture there is a progressive differentiation of the alveolar type II cell into the type I cell phenotype. In this context, we examined the production of the MMPs in cells cultured for 2 and 7 days. We

found that both type II epithelial cells and alveolar type I-like cells secreted interstitial collagenase and both gelatinases A and B. Regarding interstitial collagenase we observed a moderate decrease of collagenolytic activity in AT2 after 7 days of culture compared to AT2(2). In the present study we did not measure the levels of TIMPs in the cultures, but an increase in TIMP-1 or TIMP-2 could explain this difference.

Several cell lines that display some morphological and functional properties of alveolar type II epithelial cells have been used for different research purposes (Douglas *et al.*, 1974; Smith, 1977; Wikenheiser *et al.*, 1993). In this sense, we were interested in characterizing MMPs and comparing the cell lines to primary cultures of rat type II pneumocytes. The A549 cell line derived from a human lung carcinoma has been previously shown to produce several MMPs (Mackay *et al.*, 1992). We found that A549 and MLE-12 (mouse) cell lines exhibited, as primary cultures of type II cells, interstitial collagenase activity as well as MMP-2 and MMP-9 production. By contrast, in L2 cell line only gelatinase A was detected; an expression pattern resembling fibroblast-like gelatinase production. In addition, A549 cell line also exhibited high MW gelatinase activities that were inhibited by EDTA. These activities might represent gelatinase A and gelatinase B dimers (Lyons *et al.*, 1993; Partridge *et al.*, 1993; Lafuma *et al.*, 1994). Besides gelatinase activities we analyzed enzymatic activity on casein zymography in order to explore the possible presence of stromelysins. Regarding primary cultures of type II cells only a 92 kDa caseinolytic band was observed, similar to that revealed in alveolar macrophages. The ability of rat gelatinase B to degrade casein has also been documented by several authors (Lyons *et al.*, 1991; Partridge *et al.*, 1993). In addition, A549 and MLE-12 cell lines exhibited a 55 kDa lytic band that is compatible with stromelysin molecular mass (Matrisian, 1992).

The presence of collagenase and gelatinases A and B confers to alveolar epithelial cells the ability to degrade both stromal and basement membrane components, a capacity of considerable importance in the context of normal remodeling as well as in type II cell function during lung injury and repair. Our results support the notion that alveolar type II epithelial cells through the secretion of gelatinases A and B, could be one of the cellular sources responsible for basement membrane remodeling. Furthermore, since these cells synthesize several structural components of BM, it seems evident that they are actively participating in both pathways of basement membrane turnover. Interestingly, similar findings concerning type IV collagen breakdown have been recently reported in fetal type II epithelial cells, suggesting that these cells play a role in the genesis of basement membrane disruption during late fetal lung development (Rolland *et al.*, 1995).

Nevertheless, the production of interstitial collagenase by type II alveolar epithelial cells, raises the question of its possible biological role *in vivo*. Normally, these cells are not in direct contact with interstitial collagens. In some pathological conditions, a close relationship between type II alveolar cells and interstitial connective tissue is observed. Particularly in fibrotic lung disorders, the basement membrane is often disrupted, allowing type II alveolar epithelial cytoplasmic extensions to come in close proximity to fibroblasts and interstitial collagens (Brody *et al.*, 1981). Therefore, secretion of interstitial collagenase by these cells might play a role in the remodeling processes after lung injury. An analogous condition has been described in skin where epithelium derived from the edges of dermal wounds secretes collagenolytic activity whereas that from uninjured skin does not (Donoff *et al.*, 1971).

In addition, collagenase production by type II alveolar cells could help to prevent the intra-alveolar fibrosis occurring after acute and chronic lung damage. It has been previously reported that in a variety of lung injuries there is disruption of basement membranes with fibroblast migration to, and proliferation in, the alveolar spaces (Basset *et al.*, 1986; Fukuda *et al.*, 1990). This fibroproliferative response includes the accumulation of interstitial collagens that must be degraded in order to restore the normal parenchymal architecture. Thus, the capacity of alveolar epithelial cells to produce interstitial collagenase could represent a defensive mechanism to protect alveolar space integrity.

On the other hand, interstitial collagenase secretion by alveolar epithelial cells under normal conditions might have several putative functions. For example, if this matrix metalloproteinase is secreted by the basal surface, it could reach the interstitium and actively

participate in the normal remodeling of interstitial collagens. Likewise, if the interstitial collagenase is secreted through the apical surface, this enzyme could play a role in the physiological turnover of other possible substrates such as some surfactant proteins that have collagen-like domains (Kuroki and Voelker, 1994). However, the physiological significance of interstitial collagenase production by type II alveolar cells remains to be elucidated.

In summary, our findings demonstrate that alveolar type II epithelial cells have the capacity to synthesize *in vitro* several matrix metalloproteinases. These observations are consistent with the notion that these cells actively participate in basement membrane and extracellular matrix turnover *in vivo*. Supporting this point of view, we have recently demonstrated by *in situ* hybridization and immunohistochemistry that cells putatively identified as type II pneumocytes, are able to produce matrix metalloproteinases in acute lung injury induced by hyperoxia (Pardo *et al.*, 1996).

Acknowledgements—Supported in part by ALA Career Investigator Award to J. I. Sznajder, Michael Reese Hospital; HL-45136 to Bruce D. Uhal, Rush Medical College; CONACYT Grants # 0975P-N, 0507P-N, and F643-M9406, and PUIS, UNAM.

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