

Differential Expression of Matrix Metalloproteinases and Inhibitors in Developing Rat Lung Mesenchymal and Epithelial Cells

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ABSTRACT: Lung development requires extracellular matrix remodeling. This involves matrix metalloproteinases (MMPs) and their endogenous inhibitors [tissue inhibitors of metalloproteinases (TIMPs)]. Because these have been generally studied only in whole lung, we focused specifically on mesenchymal and epithelial cells freshly isolated at various developmental stages. In fibroblasts, the most striking developmental change was a peak (fourfold the prenatal level) of membrane type 1 (MT1)-MMP transcript during alveolarization, consistent with the known crucial role of MT1-MMP in this process. TIMP-1 and -2 mRNAs transiently increased on postnatal d (pn) 3. In alveolar epithelial cells (AECs), MMP-2 expression was maximal on fetal d (f) 19 when alveolar type II cells (ATII) differentiate and on pn5; by contrast, MT1-MMP expression changed little and TIMP-1 expression decreased with advancing gestation. In cells expressing *in vitro* the ATI phenotype, TIMP-1 and -2 activities were nine- and fivefold those in cells expressing ATII features, respectively, whereas ATII presented higher MMP-2 activity and were the only cell type to express MMP-9. This indicates higher remodeling potential for ATII. Pulmonary mesenchymal and epithelial cells have therefore quite distinct MMP/TIMP expression patterns. Changes in cell compartments should be specifically documented in developing lung diseases such as bronchopulmonary dysplasia in which changes in MMP activities have been reported. (*Pediatr Res* 62: 20–25, 2007)

Extracellular matrix (ECM) is essential to lung morphogenesis and to cell differentiation, growth, and motility (1). The successive pseudoglandular, canalicular, saccular, and alveolar stages of lung development (2) all involve remodeling of ECM (1). Zinc-dependent endopeptidases, the MMPs, carry out ECM proteolysis (3). Their activity is regulated by various mechanisms in which TIMPs play a prominent role (3).

A variety of MMPs and TIMPs are expressed in the developing lung (4,5). Although epithelial and mesenchymal cells appear to have distinct MMP/TIMP expression features (4,6), expression changes in MMPs and TIMPs across the different developmental steps have generally been studied only in the whole lung (4,5,7,8). When epithelial and mesenchymal cells were studied comparatively, it was for a very limited period

(6). The question of relative contributions is all the more important in that reciprocal epithelial-mesenchymal interactions are crucial for all lung developmental steps (9). Moreover, more attention has been paid thus far to early fetal lung development than to later stages. Further documenting late development may help to better understand the significance of changes in MMP activity associated with neonatal chronic lung disease (10,11). In the present study, we investigated expression changes in MMPs/TIMPs in fibroblasts and AECs extemporaneously isolated from the rat lung, from the end of pseudoglandular stage to alveolar stage. We also studied comparatively cells expressing *in vitro* either the ATI or ATII phenotype.

Because a global study of MMPs and TIMPs could not be considered, we focused on proteins with known particular importance, including MMP-2 and MT1-MMP (or MMP-14) that are involved in alveolar formation (12–14), TIMP-1 (the major MMP inhibitor), and TIMP-2, which controls MMP-2 activation (15). These are among the most expressed matrix-remodeling molecules in the lung, as evidenced in a profiling study in various organs of the developing mouse (5). Because we focused on late development, TIMP-3 that is important earlier during branching morphogenesis (16) was not included.

MATERIALS AND METHODS

Lung cell isolation. Dated pregnant Sprague-Dawley rats (Charles River, Saint Germain sur l'Arbresle, France) were used. Animal procedure was authorized by the French Ministry of Agriculture. AECs and fibroblasts were isolated by differential adhesion and centrifugation as described (17), on f17 (pseudoglandular stage), f19 (canalicular stage), f21, and pn1 and 3 (saccular stage), pn5 and 8 (alveolar stage, progressing septation), and pn16 (alveolar stage, terminated septation). Epithelial cells isolated by this procedure express characteristic ATII markers (17). Freshly isolated cells were immediately frozen without any culture step and stored at -80°C .

AEC culture. To determine whether cells expressing the ATI or ATII features have different MMP/TIMP equipment, AECs isolated on pn21 were seeded on substrata reported to favor either phenotype. Engelbreth-Holm-

Abbreviations: AECs, alveolar epithelial cells; ATI (II), alveolar type I (type II) cells; CF, collagen-fibronectin coating; CFL, collagen-fibronectin-laminin 5 coating; CM, conditioned medium; EHS matrix, Engelbreth-Holm-Swarm basement-membrane matrix; f, fetal day; MMP, matrix metalloproteinase; pn, postnatal day; SP-B, surfactant protein B; TIMP, tissue inhibitor of metalloproteinases

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Table 1. Sequences of primers used to amplify *TIMP-1*, *TIMP-2*, *MMP-2*, and *MT1-MMP* cDNA fragments

	Forward (5'-3')	Reverse (5'-3')	cDNA length (bp)	TM
TIMP-1	CCAGAAATCATCGAGACCACCT	GGCAGGCAAAGTGATCGCTC	420	55°C
TIMP-2	ATTATCTACACGGCCCC	CAAGAACCATCACTTCTCTTG	341	58°C
MMP-2	TGATCTCCAGTGCCCTC	TGGCGAACACAGCACTC	410	60°C
MT1-MMP	GTACTACCGCTTCAATGAGG	CACTGCCAGTACCAGGAG	363	58°C

Swarm basement membrane matrix [(EHS matrix) prepared in the laboratory] and a coating designated collagen-fibronectin-laminin 5 coating (CFL) composed of collagen I (8 $\mu\text{g}/\text{cm}^2$; BD Biosciences, Erembodegem, Belgium), fibronectin (2.1 $\mu\text{g}/\text{cm}^2$; Sigma Chemical Co., L'Isle d'Abeau-Chesnes, France), and laminin 5 (0.5 $\mu\text{g}/\text{cm}^2$; gift from P. Rousselle, Institute of Protein Biology and Chemistry, CNRS, Lyon, France), both support the ATII phenotype. Plastic and a collagen-fibronectin coating (CF), composed only of collagen I and fibronectin (same concentrations), favor transdifferentiation into ATI (18,19). Purified AECs in 10% fetal bovine serum (FBS)-containing medium were seeded (1.5×10^6 cells/mL) in multiwell plastic culture plates either uncoated or coated with one of the three matrix substrata. After overnight adhesion to substratum under air-CO₂ (95% to 5%), serum-free defined medium (17) was introduced and renewed after 24 h. After 30 h of incubation, conditioned medium (CM) was collected and stored at -80°C for MMP/TIMP assays, and the phenotype of cultured cells was determined by immunolabeling for specific markers.

Cell immunolabeling. Cytokeratin/vimentin immunolabeling allowed reciprocal contamination of AECs and fibroblasts to be determined. T1 α and surfactant protein B (SP-B) characterized ATI and ATII, respectively. Methanol-fixed cells were incubated for 1 h either with monoclonal anti-rat pan-cytokeratin antibody (Sigma Chemical Co.), monoclonal anti-rat vimentin antibody (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-rat SP-B antibody (gift from Prof. J.A. Whitsett, Cincinnati OH), or monoclonal anti-rat T1 α antibody (gift from Prof. M.C. Williams, Boston, MA), fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin antibody (Jackson ImmunoResearch Labs, Soham, Cambridgeshire, UK), or Texas red-conjugated anti-rabbit immunoglobulin antibody (GE Healthcare, Orsay, France) were used as secondary antibodies. Cell nuclei were labeled with bisbenzimidazole (Sigma Chemical Co.). Fluorescence was viewed on a Zeiss Axioskop-40 microscope.

RNA extraction. Total RNA was isolated using Trizol reagent (Invitrogen, Cergy-Pontoise, France), pelleted, dissolved in sterile water, and quantified by absorbance at 260 nm (Biospectrometer, Eppendorf). Integrity was checked by denaturing gel electrophoresis.

cDNA probes and Northern blot analysis. Total rat lung RNA was reverse transcribed using Superscript II reverse transcriptase and random hexamer primers (Invitrogen). Probes were amplified by polymerase chain reaction using target-specific primer sequences (Table 1), labeled with ($\alpha^{32}\text{P}$) deoxycytidine triphosphate (NEN, Perkin Elmer) using Rediprime labeling system (GE Healthcare), and purified on G-50 columns (GE Healthcare). For Northern blot analysis, 20 μg of RNA was electrophoresed on 1.2% agarose, 2.2-M formaldehyde gels and then transferred to nylon membranes (Gene Screen, Perkin Elmer). Membranes were successively probed for *TIMP-1*, *TIMP-2*, *MT1-MMP*, *MMP-2* mRNA, and 18S rRNA in buffer containing 50% formamide, 50 mM Tris-HCl (pH 7.5), 0.8 M NaCl, 10% dextran sulfate, 0.1% sodium pyrophosphate, 5 \times Denhardt solution, 0.1% sodium dodecyl-sulfate (SDS), and 75 $\mu\text{g}/\text{mL}$ denatured salmon sperm DNA. Hybridization was run overnight at 42°C . Blots were exposed to X-Omat AR Kodak films at -80°C . Autoradiographic signals were quantified by densitometry (NIH Image, Bethesda, MD) and normalized to 18S rRNA.

Zymography for determination of MMP-2 and MMP-9 gelatinase activities. Equal volumes of CM collected from equal number of cells were electrophoresed under nonreducing conditions on 8% SDS-polyacrylamide gels impregnated with 0.5 mg/mL of gelatin before casting. Gels were washed for 30 min in 2.5% Triton X-100 to remove the SDS, then incubated overnight at 37°C in digestion buffer (50 mM Tris-HCl and 10 mM CaCl₂, pH 7.4). Gels were stained with Coomassie blue (R250) to reveal unstained areas where the substrate was degraded by proteases. Attribution of activities to MMPs was confirmed by inhibition in the presence of ethylenediamine tetraacetic acid (10 mM). Densitometry was achieved with NIH Image software.

Reverse zymography for determination of TIMP activities. Equal volumes of CM collected from equal number of cells were electrophoresed on 13% SDS-polyacrylamide gels containing both gelatin (0.5 mg/mL) and gelatinase-rich solution produced by fetal rat lung fibroblasts stimulated by phorbol ester 12-tetradecanoylphorbol-13 acetate (20). Gels were washed and incubated for 10 h in digestion buffer at 37°C and then stained as for zymography. TIMP activities revealed as dark bands on a light background

because of gelatinase-inhibiting activity. Bands were identified by their molecular weight and comparison to a recombinant human TIMP-2 standard (gift from Prof. G. Murphy, Cambridge, UK). Densitometry was achieved with NIH Image software.

Statistical analysis. Data are presented as mean \pm SE. Multiple comparisons of mean values were made by analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD), or by nonparametric Kruskal-Wallis analysis and Mann-Whitney *U* test, depending on applicability. $p = 0.05$ was considered as the limit of statistical significance.

RESULTS

Characterization of isolated cells. AECs and fibroblasts displayed characteristic morphologic features of their cell type (Fig. 1A and C) and strongly expressed cytokeratin (Fig. 1B) and vimentin (Fig. 1D), respectively. Reciprocal contaminations were $<5\%$ of vimentin-positive cells in AECs and $<2\%$ of cytokeratin-positive cells in fibroblasts.

Developmental expression of MT1-MMP. In fibroblasts, MT1-MMP-mRNA level changed little before birth (Fig. 2A and B). It increased four- to fivefold on pn3, remained ele-

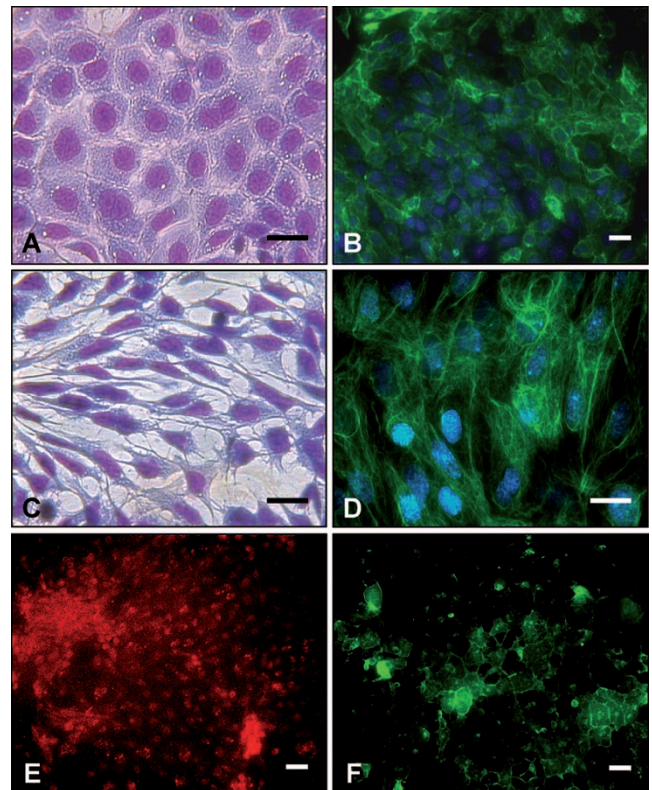


Figure 1. Characterization of isolated and cultured lung cells. Hematoxylin-eosin staining showed polygon-shaped AECs in monolayers (A), whereas fibroblasts were spindle shaped (C). AECs strongly labeled for cytokeratin (B) and fibroblasts for vimentin (D); nuclear counterstaining with bisbenzimidazole. Cells cultured on CFL matrix immunostained positively for both the ATII marker SP-B (E) and the ATI marker T1 α (F). Bars = 20 μm .

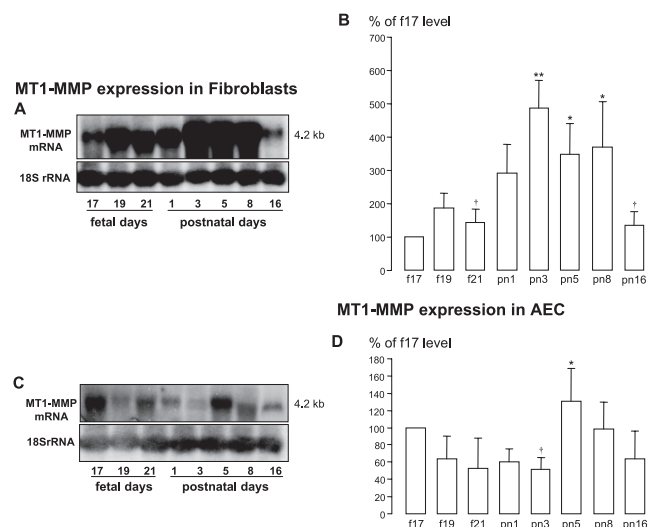


Figure 2. MT1-MMP mRNA expression in fetal and postnatal lung fibroblasts (A, B) and AECs (C, D). (A and C) Representative Northern blots. (B and D) Densitometric analysis of relative mRNA abundance normalized for loading by 18S rRNA. Mean \pm SE of five independent determinations run in five different cell preparations from different litters. Multiple comparisons by ANOVA and Fisher's PLSD. Significant difference for fibroblasts (B): * p < 0.05 vs f17; ** p < 0.01 vs f17; † p < 0.05 vs pn3 or pn8 and for AECs (D): * p < 0.05 vs pn3; † p < 0.05 vs f17.

vated until pn8, and then returned to prenatal level on pn16. In AECs, the expression level did not change significantly from f17 to pn1, but in contrast with fibroblasts, it was slightly, but significantly, decreased on pn3. A transient increase to 2.5 times the pn3 level and 1.3 times the f17 level occurred on pn5 (Fig. 2C and D). During alveolarization, comparison on same blot indicated that lung fibroblasts expressed approximately twice more MT1-MMP mRNA than epithelial cells (not shown).

Developmental expression of MMP-2. MMP-2 expression did not change significantly in fibroblasts during the period under study (Fig. 3A and B). In AECs, by contrast, it increased 50% from f17 to f19, when it was maximal coincidentally with the start of ATII differentiation; it decreased then on f21 and further decreased after birth. A slight and transient increase occurred on pn5 (Fig. 3C and D).

Developmental expression of TIMP-1 and TIMP-2. In fibroblasts (Fig. 4A and B), TIMP-1 and TIMP-2 mRNAs were rather weakly expressed on f17 as compared with later stages; they increased two- and threefold, respectively, between f17 and f19, then remained stable until pn1. For both TIMPs, a sharp and tremendous increase occurred on pn3 (four to five times the pn1 level). Expression levels decreased thereafter progressively to retrieve prenatal levels on pn8. Expression patterns of both TIMPs were strikingly similar. In AECs (Fig. 2C and D), TIMP-1 mRNA level decreased approximately 75% with advancing gestation, whereas that of TIMP-2 mRNA remained constant. Both TIMP mRNAs increased between pn3 and pn5 to reach twice the f17 level, then remained constant through pn16. Comparison of fibroblasts and AECs on same blots indicated similar levels, except on pn3, when there were higher levels in fibroblasts (not shown).

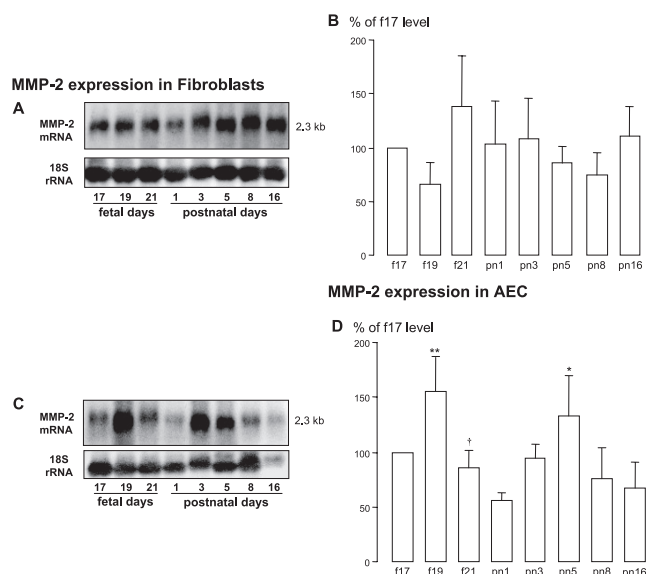


Figure 3. MMP-2 mRNA expression in fetal and postnatal lung fibroblasts (A, B) and AECs (C, D). (A and C) Representative Northern blots. (B and D) Densitometric analysis of relative mRNA abundance normalized for loading by 18S rRNA. Mean \pm SE of five independent determinations run in five different cell preparations from different litters. Multiple comparisons by ANOVA and Fisher's PLSD. Significant differences for AECs (D): * p < 0.05 vs pn1; ** p < 0.001 vs pn1; † p < 0.05 vs f19. Changes in fibroblasts did not reach significance level.

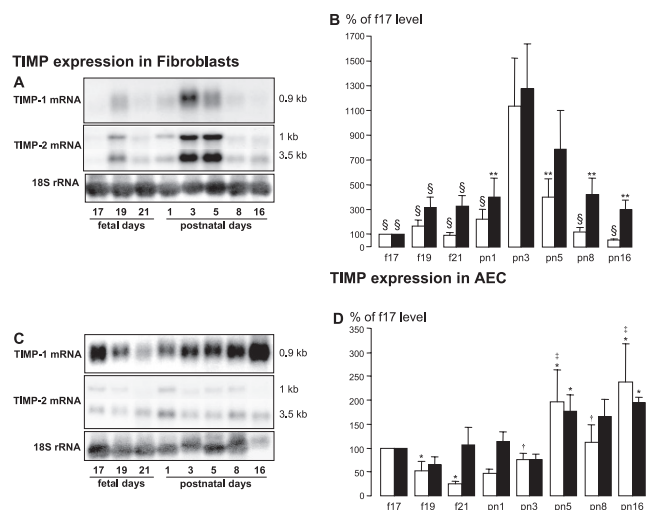


Figure 4. TIMP-1 (□) and TIMP-2 (■) mRNA expression in fetal and postnatal lung fibroblasts (A, B) and AECs (C, D). (A and C) Representative Northern blots of TIMP-1 and TIMP-2 mRNAs in fibroblasts and AECs, respectively. (B and D) Densitometric analysis of relative mRNA abundance normalized for loading by 18S rRNA. Mean \pm SE of five independent determinations run in five different cell preparations from different litters. Multiple comparisons by ANOVA and Fisher's PLSD. Significant difference for fibroblasts (B): * p < 0.05; ** p < 0.01; § p < 0.001 vs pn3, and for AECs (D): * p < 0.05 vs f17; † p < 0.05 vs f21 and pn1, ‡ p < 0.01 vs f21 and pn1.

Differential production of MMPs/TIMPs by cells expressing ATI or ATII phenotype. AECs cultured on EHS matrix massively expressed SP-B and failed to express T1 α , whereas AECs cultured on plastic or CF stained positively for T1 α , but not for SP-B (not shown). They were therefore considered as ATII and ATI, respectively. Cells cultured on CFL simultaneously expressed SP-B and T1 α (Fig. 1E and F), therefore

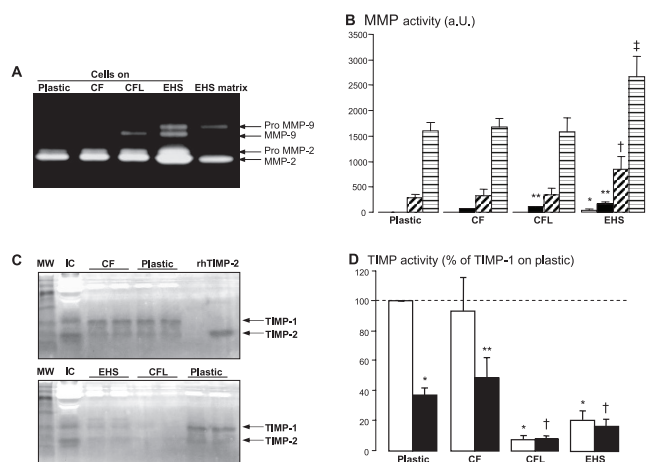


Figure 5. Comparison of gelatinase (MMP-2 and -9) and TIMP-1 and -2 activities released in CM by AECs cultured on different substrata. (A and B) Gelatinase activities evaluated by zymography. (A) Representative zymogram of CM from cells on plastic, CF, CFL, or EHS matrix, and from EHS matrix without cells. Gelatinolytic activities appear as strong bands for MMP-2 (▨) and weaker bands for pro-MMP-2 (▨), pro-MMP-9 (□), and MMP-9 (▩). (B) Densitometric analysis (arbitrary units, a.u.); activity from EHS matrix alone was subtracted from total activity to determine the part due to AECs cultured on this substratum. (C and D) TIMP activities evaluated by reverse zymography. (C) Representative reverse zymograms of CM from cells on CF, plastic, EHS, and CFL. MW, molecular weight markers; IC, internal control (Dulbecco modified Eagle medium-10% FBS); rhTIMP-2, recombinant human TIMP-2 used as positive control. Gelatinolytic-inhibiting activities appeared as zones of positive staining with Coomassie blue, with two major areas at 28 (TIMP-1; □) and 21 kD (TIMP-2; ■). (D) Densitometric analysis. Data are mean \pm SE of three independent determinations (different cell culture experiments). Multiple comparisons by Kruskal-Wallis analysis and Mann-Whitney *U* test. Significant difference in B: * $p < 0.001$ vs pro-MMP-9 on plastic; ** $p < 0.001$ vs MMP-9 on plastic; † $p < 0.001$ vs pro-MMP-2 on plastic; and ‡ $p < 0.01$ vs MMP-2 on plastic. Significant difference in D: * $p < 0.001$ vs TIMP-1 on plastic; ** $p < 0.01$ vs TIMP-1 on CF; † $p < 0.01$ vs TIMP-2 on CF.

displaying an ATI-ATII intermediary phenotype. As shown in Figure 5A and B, cells produced similar MMP-2 amounts on all substrata, except on EHS matrix where the activity was 45% higher. Only cells grown on EHS matrix or CFL released MMP-9, although in much lower proportion than MMP-2. Cells on CFL displayed secretion features intermediary between those of cells on CF and EHS matrix. As depicted in Figure 5C and D, TIMP-1 activity from cells on plastic or CF was approximately nine- and fivefold higher than that from cells on CFL and EHS matrix, respectively, and threefold greater activity was observed for TIMP-2. On plastic and CF, TIMP-1 activity was about twice TIMP-2 activity, whereas on EHS matrix or CFL, both TIMPs presented similar levels (Fig. 5D). The MMP/TIMP balance therefore appears in favor of greater proteolytic activity for cells on CFL or EHS matrix.

DISCUSSION

The MMP/TIMP system plays a key role in epithelial-mesenchymal interactions that are essential for lung development. We profiled the expression of key members of the MMP and TIMP families in lung fibroblasts and AECs isolated at various stages, and tentatively correlated variations with morphologic changes. We demonstrated quite different patterns

among cell types, which shows that previously reported changes in whole lung were not informative enough to understand all developmental implications.

Using isolated cells is a possible limitation of this study because we cannot rule out the possibility that cell isolation might have altered transcript levels to some extent. The study, however, allowed developmental changes to be evidenced that are unlikely to be due to cell processing. Similarly, the low reciprocal contaminations of fibroblasts and AECs are unlikely to have altered the results because changes in one cell type were not reflected in the other. For instance, the marked increase in MT1-MMP or TIMP mRNA levels in fibroblasts on pn3 went along with no change or a decrease in AECs. Such possible bias would indeed be avoided by *in situ* hybridization or immunohistochemistry, but these approaches can hardly provide precise quantitative determination and are no more exempt of limitations. Thus, they sometimes led to contradictory results, with either no expression of MMP-9 in fetal rat lung (12) and of TIMP-1 in fetal rabbit lung (7) or, conversely, expression of MMP-9 in fetal rat (21) or monkey (11) lung and of TIMP-1 in monkey (11) or human and mouse (8) lung. Another limitation resides in the impossibility to determine MMP/TIMP activities in freshly isolated cells because collecting medium with released material implies several hours of culture, which may have induced still greater changes than cell isolation. Except in the instance of ATI/ATII comparison, we had therefore to limit our investigation to the pretranslational level and to infer from expression changes for putative changes in activity. Last, although we previously showed that AECs isolated by this procedure expressed specific ATII markers (17), we cannot exclude that part of cells herein designated AECs were in fact Clara cells that also express SP-B. This, however, was unlikely to interfere with our primary finding of differences between epithelial cells and fibroblasts.

A major finding consisted in localizing considerable changes in MT1-MMP expression to fibroblasts during alveolarization, which was not evident from previous global profiling studies (5) or examinations of expression including *in situ* hybridization in mice (13,14). In addition, the lack of a relationship among MMP-2, TIMPs, and MT1-MMP was unexpected based on previous whole-lung profiling data (5). Alveolarization comprises a period of alveolar-sac proliferation that occurs before birth, a period of secondary septation that takes places from 4 to 14 pn in the rat, and finally a period of wall thinning and microvascular maturation with fusion of the double capillary network into a single network (2). Peak expression of MT1-MMP in fibroblasts between pn3 and pn8, followed by decrease on pn16, suggests a link with septation process. Moreover, although much less striking, maximal level was also achieved in AECs during the same period, on pn5. Previous findings from *in situ* hybridization in mice indicating peak expression in alveolar walls around d 10 are also consistent with this hypothesis (14). High MT1-MMP expression in this period is in keeping with its essential role in alveolar development recently demonstrated by the lack of septation in MT1-MMP-deficient mice (13,14). Although other cell types including endothelial cells could also contrib-

ute, our findings argue in favor of a major contribution of fibroblasts in producing MT1-MMP during alveolarization. With regard to its mechanism of action, although MT1-MMP is known to activate pro-MMP-2 (22), its role in alveolarization appears to be largely independent from this ability (13,14). Consistently, MMP-2-null mice have only limited alveolarization defects (12,13), and we did not find significant changes in MMP-2 expression in fibroblasts during lung development. Taking into account the cell motility-promoting and invasion-promoting activities of MT1-MMP (23), on the one hand, and the requirement of myofibroblast migration for elastin deposition and alveolarization (24) on the other, an essential role of MT1-MMP in myofibroblast migration to locations where secondary septa surge is a likely hypothesis. Because MT1-MMP favors neovessel formation (25), it may also be crucial for microvascular development (13), an essential component of alveolarization.

In contrast with fibroblasts, MMP-2 expression in AECs fluctuated with development, which is likely to have functional significance. A first peak of expression on f19 presumably correlated with formation of basement membrane (BM) discontinuities underneath ATII through which foot processes protrude and establish contact with lipofibroblasts (26,27). ATII maturation correlates with increased frequency of those BM discontinuities that appear essential to epithelial-mesenchymal interactions (26,27). Interestingly, our investigation also demonstrated that ATII present higher matrix proteolytic potential than ATI, which is likely to be in connection with the preferential association of membrane discontinuities with the former. In agreement with our findings, AECs cultured on plastic presented higher collagenolytic activity after 2 d of culture than after 7 d (28), which probably reflects conversion from ATII to ATI phenotype on this substratum. Moreover, the decreased MMP-2 expression observed in AECs between f19 and f21 (Fig. 4) may reflect the increased proportion of ATI between both stages (2). After a decrease around birth, a second peak of MMP-2 expression was observed on pn5. Its significance is unclear. It does not seem to relate to BM discontinuities that regularly decrease after birth (27), and, contrary to the peak of MT1-MMP expression in fibroblasts, it was not maintained throughout alveolar septation. Nevertheless, it was reported that infants evolving toward bronchopulmonary dysplasia present decreased MMP-2 and increased MMP-9 activities in tracheal effluent (10,11). These changes are likely to represent altered release by epithelial cells. Because alveolarization is impaired in the disease, they suggest functional importance of elevated epithelial MMP-2 expression when alveolarization starts, although its precise role is unknown.

TIMP-1 expression was found to be minimal in both fibroblasts and AECs at the end of gestation and to diminish with maturation in AECs. During the corresponding canalicular and saccular stages, air-blood barriers appear and develop, matrix material reduces, and foot processes increase in number. Assuming reflected changes in activity, a low TIMP-1 level may favor proteolytic activity necessary to degrade ECM during these processes. Although striking, the expression peak of TIMP-1 and -2 in fibroblasts on pn3 may, by contrast, not

be related to a particular developmental event because it occurs at a time when the lung presents little change after prenatal surfactant accumulation and before the start of alveolarization. Minoo *et al.* (29) previously reported similar TIMP-1 mRNA increase in neonatal baboon whole lung after premature or term birth. Its presence in premature neonates led to consider this increase as a consequence of passage to oxygen breathing (29). Our findings are likely to represent the same phenomenon. The significance of this transient increase is unclear, but whether it was reflected in higher TIMP activity, it seems likely to be associated with limited postnatal ECM remodeling. Postnatally, both TIMP-1 and -2 transcripts increased progressively in AECs and stayed maximal between pn5 and pn16. In fibroblasts, by contrast, TIMP-1 and -2 expression declined from pn5 to pn8 and reached a particularly low level on pn16. High levels in AECs may correspond to progression and stabilization of BM with decreased frequency of discontinuities (27). Relatively high expression level of TIMPs in both compartments during septation may also facilitate secondary crest formation by limiting degradation of soluble elastin (tropoelastin) before stabilization by cross-linkage. Last, since TIMPs promote cell growth independently of their MMP-inhibiting effects (30,31), high TIMP levels in postnatal weeks may contribute to the intense cell proliferation observed for all cell types at this time (2). Reciprocally, decreased TIMP expression in fibroblasts after completion of septation may correlate with thinning of the interstitial compartment that occurs in this last developmental stage (2).

As a whole, this study brings new insights into MMP/TIMP expression in the developing lung, pointing out the importance of distinguishing between different cells. In the perspective of preventing diseases that include changes in MMP activities, particularly bronchopulmonary dysplasia, changes in various cell compartments should be documented.

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