Virchows Arch (1996) 428:207–215

ORIGINAL ARTICLE

M. Kasper · P. Lackie · M. Haase · D. Schuh
M. Müller

Immunolocalization of cathepsin D in pneumocytes of normal human lung and in pulmonary fibrosis

Received: 14 November 1995 / Accepted: 25 March 1996

Abstract Cathepsin D expression has been assessed by immunohistochemistry and immunoelectron microscopy in fetal, normal adult and injured lungs of human beings. In addition to the well known positivity of alveolar macrophages and the bronchial epithelial cells, normal type I and to a lesser extent type II pneumocytes showed a granular, cytoplasmic staining pattern. Using immunogold labelling of lowicryl embedded human lung, cathepsin D was present in lysosomes of epithelial cells. Double immunofluorescence labelling employing type I and type II specific antibodies or lectins confirmed the epithelial staining for cathepsin D. At the terminal sac period during lung development cathepsin D appears in the alveolar epithelium. In fibrotic specimens, enhanced immunoreactivity was found in epithelial and non-epithelial cells. Proliferative epithelial formations were strongly stained with cathepsin D antibodies, whereas detached, desquamated epithelial cells were weakly positive or negative. We suggest that cathepsin D plays a role in the remodelling process during fibrogenesis.

Key words Cathepsin D · Pulmonary fibrosis · Alveolar epithelium

Introduction

The aspartic proteinase cathepsin D (EC 3.4.23.5) is an ubiquitous enzyme with optimum activity under acidic (PH 2.8–4.0) conditions. The enzyme is primarily located within lysosomes and in membranes of macrophage endosomes. The main functions of this enzyme are the catabolic degradation of intracellular proteins, the activation of other proteolytic enzymes, the stimulation of DNA synthesis and mitosis during tissue regeneration as well as the direct degradation of extracellular matrix components (reviewed in [16]). In this context, recent experimental observations have shown that some pathological processes related to inflammation such as liver cirrhosis, myocardial injury, wound healing of skin and interstitial pulmonary diseases are associated with increased activity of cathepsin D [8, 21, 27]. Furthermore, cathepsin D has been implicated in the process of tumour invasion and metastasis [16]. In normal lung tissue, cathepsin D is expressed in the lysosomes of alveolar macrophages and in secretory granules of bronchial epithelial cells [1, 3]. The first report on the cathepsin D distribution in lung tissue also described the occurrence of cathepsin D in alveolar lining cells of human lung [19]. However, more recent studies have not supported this observation [1, 3].

In the present immunocytochemical investigation, the localization of cathepsin D in human lung has been reevaluated at the light microscopic and at the ultrastructural level using samples from normal (fetal and adult)

M. Kasper (✉) · M. Haase · D. Schuh · M. Müller
Institute of Pathology, Technical University of Dresden,
Fetscherstrasse 74, D-01307 Dresden, Germany
Tel.: (49) 351-458 3023, Fax: 351-458 4328

P. Lackie
Southampton General Hospital, University of Southampton,
Southampton SO16 6YD, UK

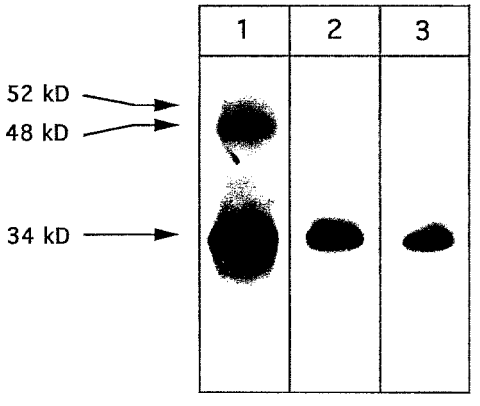

Fig. 1 Western blot analysis of antibodies to cathepsin D in human lung homogenate. *Lane 1* Monoclonal antibody OS13A to human cathepsin D (Becton Dickinson), *lane 2* polyclonal antibody to human cathepsin D (Biogenex), *lane 3* polyclonal antibody to human cathepsin D (Oncogene Science)

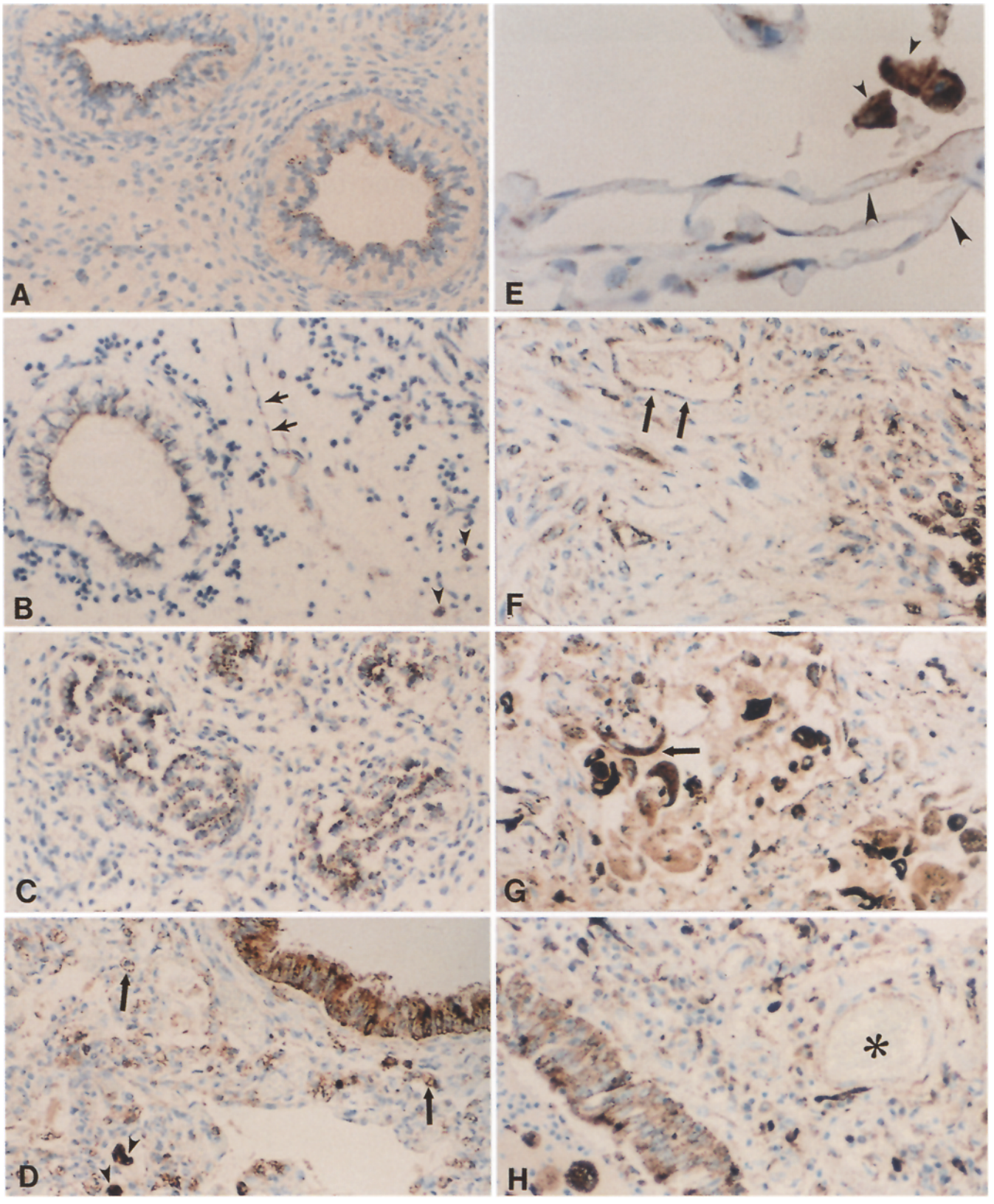

Fig. 2A–F Paraffin sections of human lung tissues. Immunoperoxidase demonstration of cathepsin D in fetal (**A** embryonic stage, **B** pseudoglandular stage, **C** canalicular stage, **D** saccular stage), adult (**E**) and fibrotic (**F–H** three different cases) using the monoclonal antibody OS13A. Note the increasing immunoreactivity during lung development. In **D** first staining of alveolar epithelial cells appeared (*arrows*). *Small arrowheads* in **B** and **D** indicate immunopositive alveolar macrophages. Note some immunopositivity in endothelial cells (**B** *Small arrows*). In the adult lung (**E**) note a weak staining of the alveolar lining cells (*arrowheads*) and a strong staining of alveolar macrophages (*small arrowheads*). Fibrotic tissues show additional immunoreactivity for cathepsin D in interstitial cells (**F**) in the tubular alveoli (**F** *arrows*), in desquamated epithelial cells (**G** *arrow*) and in some endothelia (**H** *asterisk*). **A–D, F–H** ×150; **E** ×300

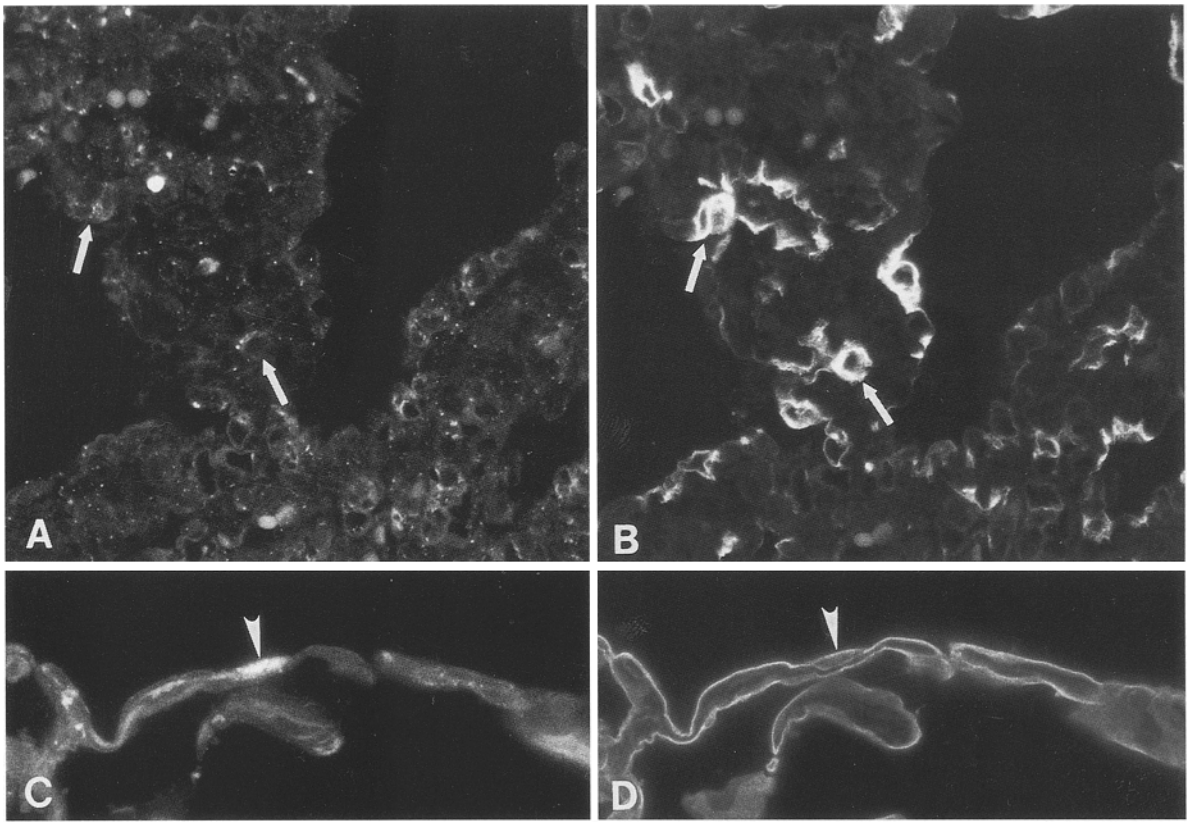

Fig. 3A–D Paraffin sections of normal human lung. Double immunofluorescence demonstration of cathepsin D (**A, C**) and cytokeratin (**B**) or lectin *Lyopersicon esculentum* (**D**) in fetal (saccular stage; **A, B**) and adult (**C, D**) tissue. Note the presence of cathepsin D in fetal alveolar epithelium (**A, B** *arrows*) and in a flat type I pneumocyte (**C, D** *arrowhead*). **A–D** ×300

and pathological conditions. For this purpose we used four different polyclonal and monoclonal antibodies to cathepsin D to study the distribution of this proteinase in tissues with pulmonary fibrosis.

Materials and methods

Fibrotic pulmonary specimens were obtained from twelve autopsies 8–10 h post mortem and from biopsies of four cases of sarcoidosis. Samples with pulmonary fibrosis were derived from five patients with primary bronchial carcinoma metastases, three patients with mammary carcinoma metastases and from one patient each with renal cell carcinoma metastases, lymphogranulomatosis, osteosarcoma and seminoma. All were male patients, except those with breast cancer; (for patient data see our previous, study, [15]). The patients developed diffuse pulmonary fibrosis, but in cases which had received radiotherapy only the fibrotic parts of the lung were investigated.

Normal adult lung tissue was obtained from two individuals after lobectomy due to primary lung cancer. Samples were taken distal to the tumour in macroscopically normal areas. The eight human embryos and fetuses were obtained following abortion. Clinical data on gestational age were known and crown-rump length was measured (7, 9, 10, 12, 16, 17, 21 and 34 weeks of gestation).

For routine histological examination, the samples were fixed in 4% formaldehyde, embedded in paraffin, and stained with haematoxylin and eosin.

The following antibodies were used: polyclonal rabbit antiserum to cathepsin D, specific for 34 kDa mature cathepsin D protein (Ab-2; Dianova, Hamburg, Germany); polyclonal rabbit antiserum to cathepsin D, specific for the 52 kDa and 34 kDa forms (Biogenex, Hamburg, Germany); monoclonal mouse antibody to cathepsin D, clone OS13A (Becton Dickinson, Heidelberg, Germany), dilution 1:100; monoclonal mouse antibody to cathepsin D, clone C5 (Medac, Hamburg, Germany), dilution 1:200; polyclonal rabbit antibody to keratin (code number Z622; Dakopatts, Hamburg, Germany), dilution 1:100.

For immunohistochemistry paraffin sections were mounted on slides that were coated with silane. After drying overnight, the sections were dewaxed, and irradiated with microwave in 0.01 M sodium citrate buffer (pH 6.0), 2×5 min at 750 W. After washing in phosphate buffered saline (PBS; pH 7.4) for 5 min, the sections were treated with 0.3% hydrogen peroxide for 30 min, incubated with 30% fetal calf serum and then incubated with the rabbit polyclonal antisera against cathepsin D, dilution 1:20. The antibodies were detected by the use of peroxidase-coupled goat anti rabbit IgG [horse radish peroxidase (HRP) 77, Dr. H. Großmann; dilution 1:400]. The peroxidase activity was visualized with diaminobenzidine. Negative controls included omission of the primary antibody and its replacement by PBS or normal rabbit serum. The monoclonal mouse antibodies were detected using the avidin-biotin peroxidase complex technique (Vectastain Elite kit, Vector Laboratories, Burlingame, Calif., USA) according to the suggested protocol. The dilution of monoclonal antibodies was 1:10 after microwave treatment.

For double fluorescence labelling paraffin sections were microwaved, washed in PBS and incubated with fluoroscin isothiocyanate-labelled *Maclura pomifera* lectin (MPA; Medac, Hamburg, Germany), diluted 1:200, followed by incubation with the monoclonal antibody to cathepsin D OS13A and Texas Red-labelled goat anti mouse immunoglobulin (dilution 1:80; Dianova). Other double staining was performed using the polyclonal anticytokeratin antiserum Z622 and the monoclonal antibody OS13A (for methods of double staining see [13]). All antibodies were diluted in PBS unless otherwise stated. As negative controls we re-

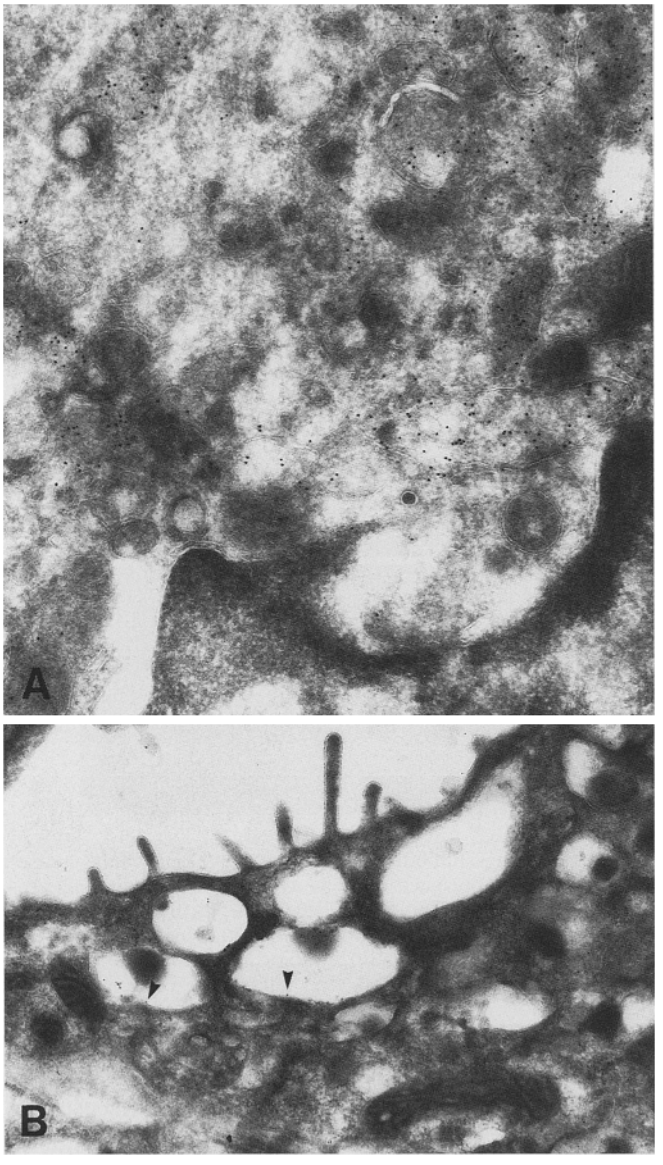

Fig. 4A, B Ultrathin sections of normal lung prepared by cryoultramicrotomy. Immunogold staining for cathepsin D using monoclonal antibody OS13A in a type I cell (**A**) and in a type II cell (*arrowheads*, **B**). **A** ×40,000, **B** ×23,000

placed the primary antibodies with PBS, rabbit non-immune serum or irrelevant monoclonal IgG. MPA binding (sugar specificity: α-D-GalNAc>αGal) was inhibited by a 0.2 M solution of the corresponding inhibitory sugar methyl-alpha-D-galactopyranoside.

Since insufficient samples were available to use a single protocol for immunoelectron microscopy, specimens were processed in one of two ways. To provide blocks from which ultrathin sections could be cut, samples after fixation were either embedded in Lowicryl resin or frozen. Processing by these different protocols (detailed below) provides further confirmation of the specificity of the cathepsin D immunolocalization.

For cryosectioning of normal human lung, the method of Tokuyasu et al. [24, 25] was followed. Briefly, small samples of lung were infiltrated with cryoprotectant containing 2.3 M sucrose and 20% 10 kDa polyvinylpyrolinidone in PBS for at least 4 h and then frozen in liquid nitrogen. Ultrathin sections (50–120 nm) at approximately −110° C using a Ultracut F4 cryoattachment (Leica, Milton Keynes, UK). These sections were transferred onto formvar coated grids and stored on 2% gelatine plates which were liquified at 37° C before immunostaining with monoclonal antibody OS13A. Ten nanometer gold labelled goat anti mouse secondary antibody (Sigma) was used at O_{525}=0.1 in PBS containing 1% bovine serum albumin (BSA), 0.05% Triton X100 and Tween 20. Sections were postembedded in methyl cellulose containing 0.2% uranyl acetate following the method of Griffiths et al. [7] before viewing.

Furthermore, tissue pieces (about 1–2 mm^3) from a lung biopsy obtained from a patient with idiopathic interstitial fibrosis were embedded in Lowicryl HM20 and immunostained as previously described [14]. Briefly, paraformaldehyde-fixed samples were frozen in liquid nitrogen, cryosubstituted and embedded in Lowicryl (through a graded series of methanol). Ultrathin sections were mounted on nickel grids, previously coated with 3% colloidin. The grids were incubated with 10% normal goat serum in buffer [TRIS-buffered saline (TBS), pH 7.6] for 45 min, and then with anti cathepsin D antibodies (monoclonal antibody OS13A and the polyclonal antibody from Biogenex; concentration 10 fold higher than used for immunohistochemistry) overnight at 4° C and for 1 h at room temperature (RT). After washing with buffer (TBS containing 0.2% BSA) sections were incubated with 40 nm gold-conjugated goat anti rabbit IgG or with 10 nm gold-conjugated goat anti mouse (1:50 in TBS, pH 8.2) for 1 h. After further washing with buffer, the sections were stained with 2.5% uranylacetate in 50% ethanol and 1% lead citrate for 2 min each.

For western blotting 1 g of deep-frozen human lung tissue was suspended in 5 ml of homogenization buffer (63 mM Tris-hydrochloric acid, pH 6.8, 8.5% glycerol, 2% sodium dodecyl sulphate (SDS), 5% 2-β-mercaptoethanol and 0.125% bromphenol blue) and homogenized with an ultra-thurrax for 1 min. The sample was then heated to 95° C for 4 min and centrifuged at 30,000 *g* for 30 min. The supernatant was filtered through a membrane filter (0.45 µm) and stored at 4° C. Twenty micrograms of protein was separated on a 10% SDS polyacrylamide gel and blotted onto an ECL nitrocellulose membrane (Amersham). After blocking with 5% fat-free dry milk, the membranes were incubated with rabbit polyclonal antisera to cathepsin D [supplied by Dianova (Ab-2) 1 µg/ml and by Biogenex, 2 µg/ml], mouse monoclonal antibody to cathepsin D (Becton Dickinson, 1 µg/ml) for 1 h at RT, and after washing with the second antibody (rabbit Ig HRP linked whole antibody, Amersham, dilution 1:3000) for 1 h at RT. Peroxidase labelled antibodies were visualized using the ECL western blotting reagent (Amersham) according to the manufacturer's instructions.

Results

Western blot analysis clearly showed a band of 34 kDa indicating that cathepsin D is present in normal human lung homogenate (Fig. 1).

Cathepsin D was seen at early stages of development in the apical part of the lung primordium (Fig. 2A). At later stages of development (pseudoglandular and canalicular stages), some interstitial and endothelial cells were labelled (Fig. 2B, C). The saccular stage was characterized by the appearence of immunopositivity in the developing alveolar epithelium and by prominent staining of alveolar macrophages (Fig. 2D). Double label immunofluorescence employing keratin as an epithelial marker confirmed the epithelial distribution of the cathepsin D immunostaining at this stage of development (Fig. 3A, B). In the adult lung cathepsin D was present in the bronchial epithelium, in the alveolar macrophages and weakly in the alveolar epithelial cells (Fig. 2E, Fig. 3C, D).

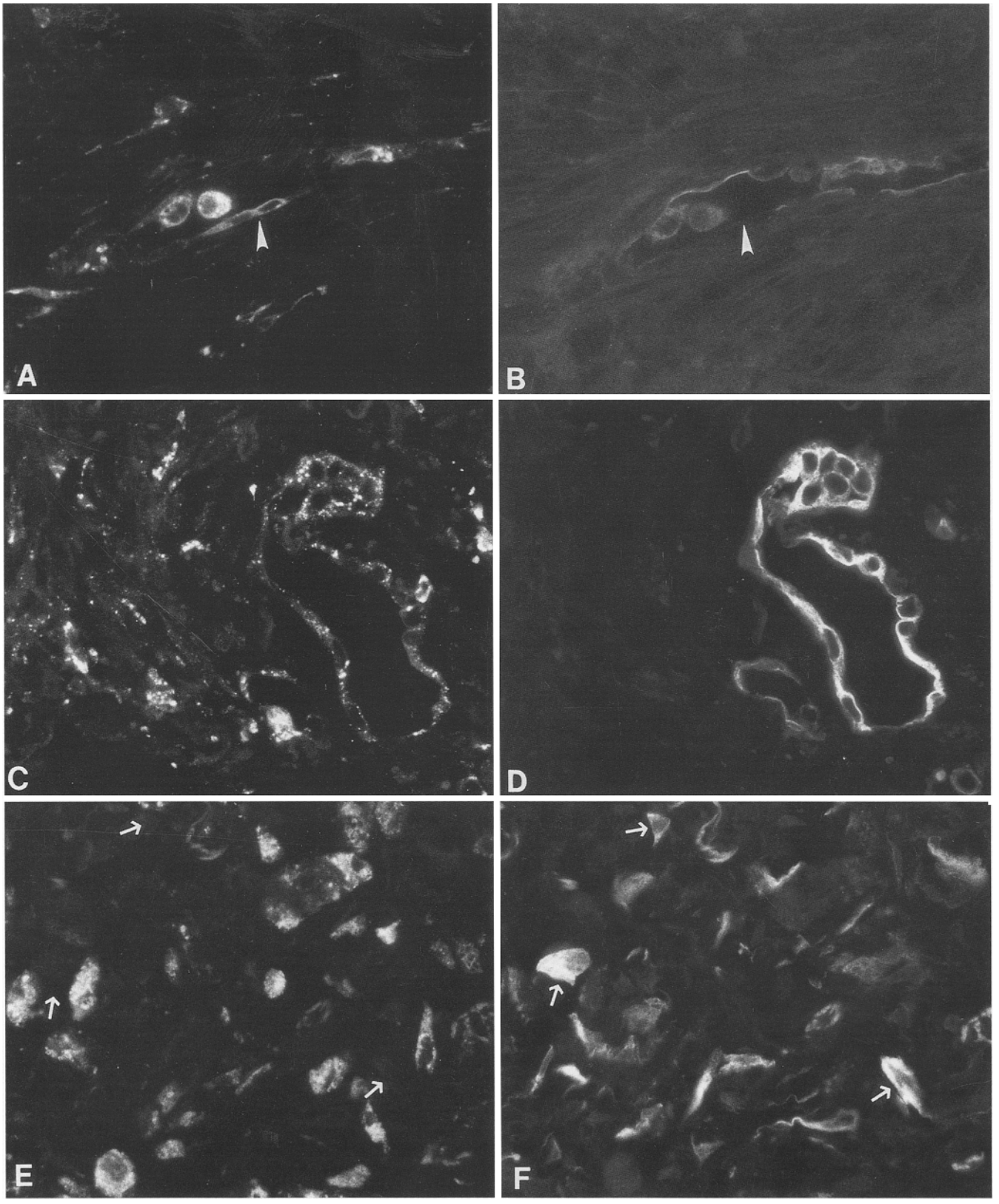

Fig. 5A–F Paraffin sections of fibrotic lung tissue (for **A–D**, compare with Fig. 2F; for **E–F**, compare Fig. 2G). Double immunofluorescence demonstration of cathepsin D (**A, C, E**) and *Maclura pomifera* (MPA; **B**) or cytokeratin (**D, F**). Strongest cathepsin D was found in MPA-negative type I alveolar epithelial cells (**A** *arrowhead*). Note the strong cathepsin D expression in tubular alveoli (**C**), whereas detached pneumocytes in another case (**E, F** *small arrows*) were devoid of staining. **A–F** ×300

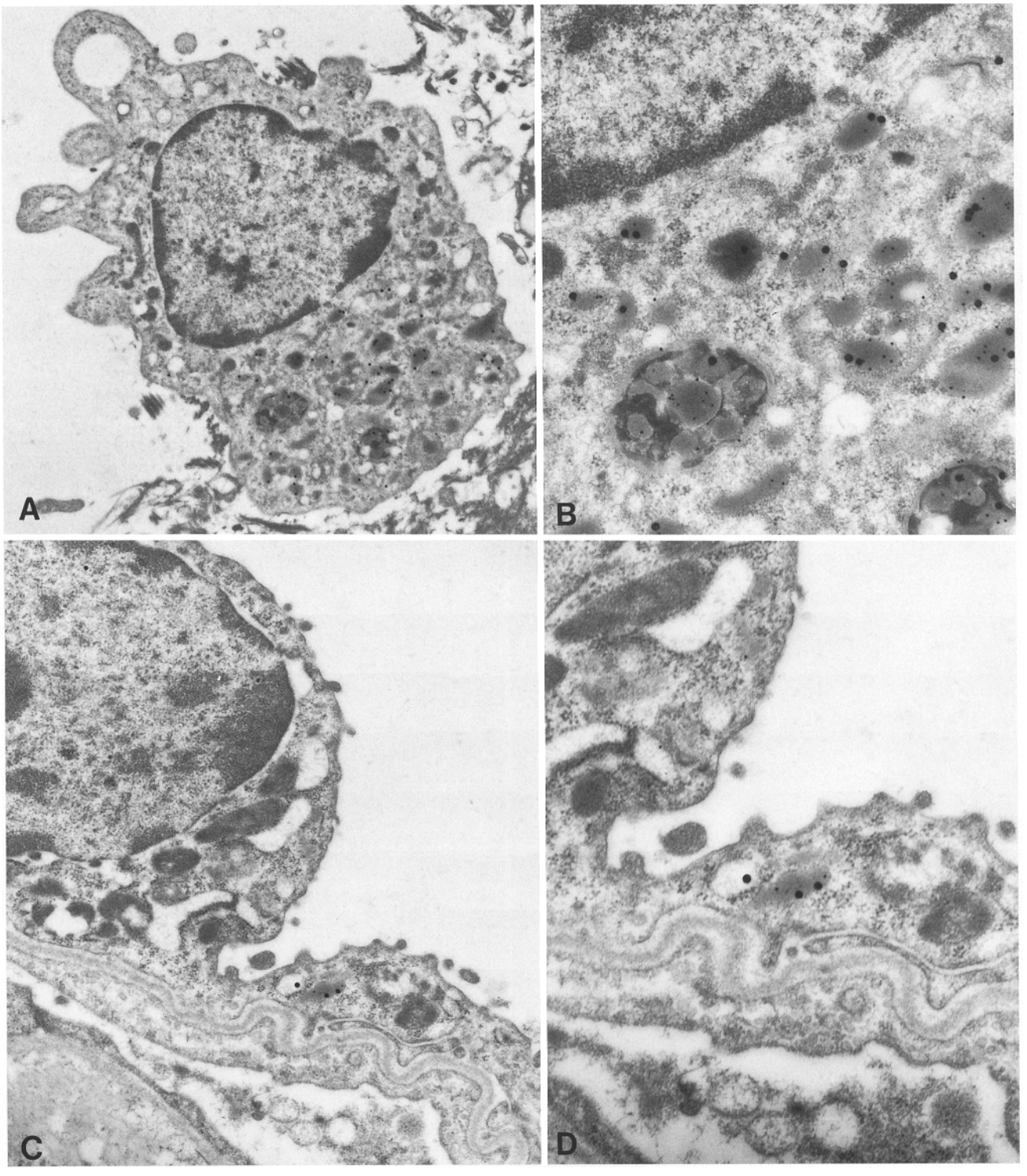

Fig. 6A–D Lowicryl HM20 embedded human lung (pulmonary fibrosis). Double immunogold staining of an alveolar macrophage (**A, B**) and a type I cell (**C, D**) for cathepsin D using a polyclonal antiserum (40 nm gold) and the monoclonal antibody OS13A (10 nm gold). **B, D** Higher magnification of **A, C**. **A** ×10,500; **C** ×18,000; **B, D** ×31,500

Using immunogold labelling on ultrathin frozen sections, cathepsin D was present in type I and II pneumocytes (Fig. 4).

Tissue sections of fibrotic lungs were stained for four different polyclonal and monoclonal antibodies against cathepsin D. Typical examples of immunostaining of cathepsin D are shown in Fig. 2F–H. Light microscopic investigation showed signs of end-stage disease including lesions of fibrotic and obliterated alveoli covered by pro-

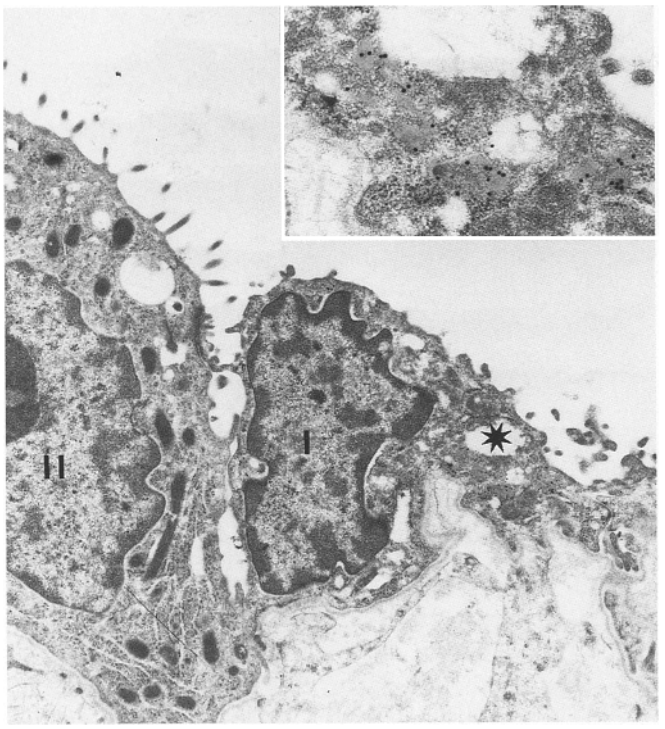

Fig. 7 Part of the human lung from the same patient showing relatively normal alveolar epithelium despite the thickening of the underlying extracellular matrix. Immunogold demonstration of cathepsin D in a type I cell (*I*) neighbouring a negative type II pneumocyte (*II*). *Inset* shows higher magnification of area indicated by an *asterisk*. ×10,500; *inset* ×31,500

liferative or cuboidal and metaplastic epithelium and associated with increased numbers of alveolar macrophages. At some sites, a clear-cut distinction between type I and II epithelial cells was no longer possible. There were no tumour cells or metastases in the samples used for immunostaining. Staining of alveolar macrophage clusters, interstitial cells (particularly of myofibroblasts), some endothelial cells (Fig. 2F, H) and of the remodelling alveolar epithelium (Fig. 2F–H) was observed in all cases. The results obtained with both monoclonal and polyclonal antibodies to cathepsin D corresponded.

Double immunofluorescence for cathepsin D and the type II cell marker MPA, a lectin which does not bind to type I alveolar epithelial cells [6], revealed stronger staining of MPA-negative type I alveolar epithelial cells in proliferative tubular alveolar epithelium (Fig. 5A, B). Double staining with keratin showed some specimens with strongest cathepsin immunoreactivity in the alveolar epithelium (Fig. 5C, D; compare with immunostaining of Fig. 2F) but in samples with complete detachment of the epithelial cells (Fig. 5E, F; compare Fig. 2g) there was very weak cathepsin D staining.

By using immunoelectron microscopy from the lung of the case shown in Fig. 2F, available from technical reasons only as a Lowicryl-embedded sample, cathepsin D was mainly localized in lysosomes of alveolar macrophages and in perinuclear lysosomes of type I alveolar epithelial cells but not or seldom in the organelles of flat type I cell processes (Figs. 6, 7). Double immunogold labelling with a polyclonal and a monoclonal antibody showed the colocalization of both antibodies in the same organelles (Fig. 6). In contrast, type II pneumocytes were weakly and focally decorated with gold particles (Fig. 8A, B). In proliferative parts of the alveolar epithelium, however, greater accumulation of immunopositive organelles was detectable (Fig. 8C, D). More scarcely, a weak staining of myofibroblasts was found (not shown).

In cases with sarcoidosis, enhanced cathepsin D expression was also found in fibroblasts, macrophages, endothelial cells and in hyperplastic type II pneumocytes (not shown).

Discussion

The present investigation emphasizes two aspects of cathepsin D expression in human lung. This study is the first to confirm convincingly the expression of cathepsin D in the alveolar epithelium of fetal and adult human lung; previous studies have shown that pulmonary tissue alveolar macrophages and the bronchial epithelium are the main source of lysosomal activity [1, 3]. To investigate whether cathepsin D plays a role in fibrogenesis in the human lung, we examined cathepsin D expression under pathological conditions in pulmonary fibrosis. We demonstrated that high cathepsin D immunoreactivity was observed in the regenerating or remodelling alveolar epithelium. However, also cases with sarcoidosis where fibrotic alterations are caused by the infiltration of inflammatory cells, showed enhanced immunoreactivity in the lung parenchyma.

The enhanced immunoreactivity for cathepsin D in alveolar macrophages and in the alveolar epithelium as shown in this study is in line with the established activation of proteases during fibrogenesis. Evidence has accumulated that proteases, which are also produced by the alveolar epithelium, may be responsible for proteolysis of the surrounding extracellular matrix. Tissue degradation and remodelling in lung injury during early and late stages of pulmonary fibrosis are regulated by the action of proteolytic enzymes such as metalloproteases, serine proteases, aspartyl proteases (such as cathepsins D, E and pepsinogen II) and cysteine proteinases (such as cathepsins B, S, H and L). Two aspartatic proteinases, pepsinogen II and cathepsin E have already been identified by immunocytochemistry in type II pneumocytes and bronchiolar Clara cells, respectively. Epithelial hyperplasia in interstitial lung diseases was accompanied by enhanced expression of both proteinases [3]. There is also evidence that cathepsin D is a physiological regulator of the other cathepsin-B and L-like cysteine proteinases [22]. The cysteine proteinase cathepsin B is involved in extracellular matrix processing of epithelial lung tumour cells [9, 23, 26] and in in vivo degradation of rat lung collagen [4]. Cathepsin H has been found in normal type II pneumocytes and alveolar macrphages [10]. Under pathological conditions cathepsin B was found in myofi-

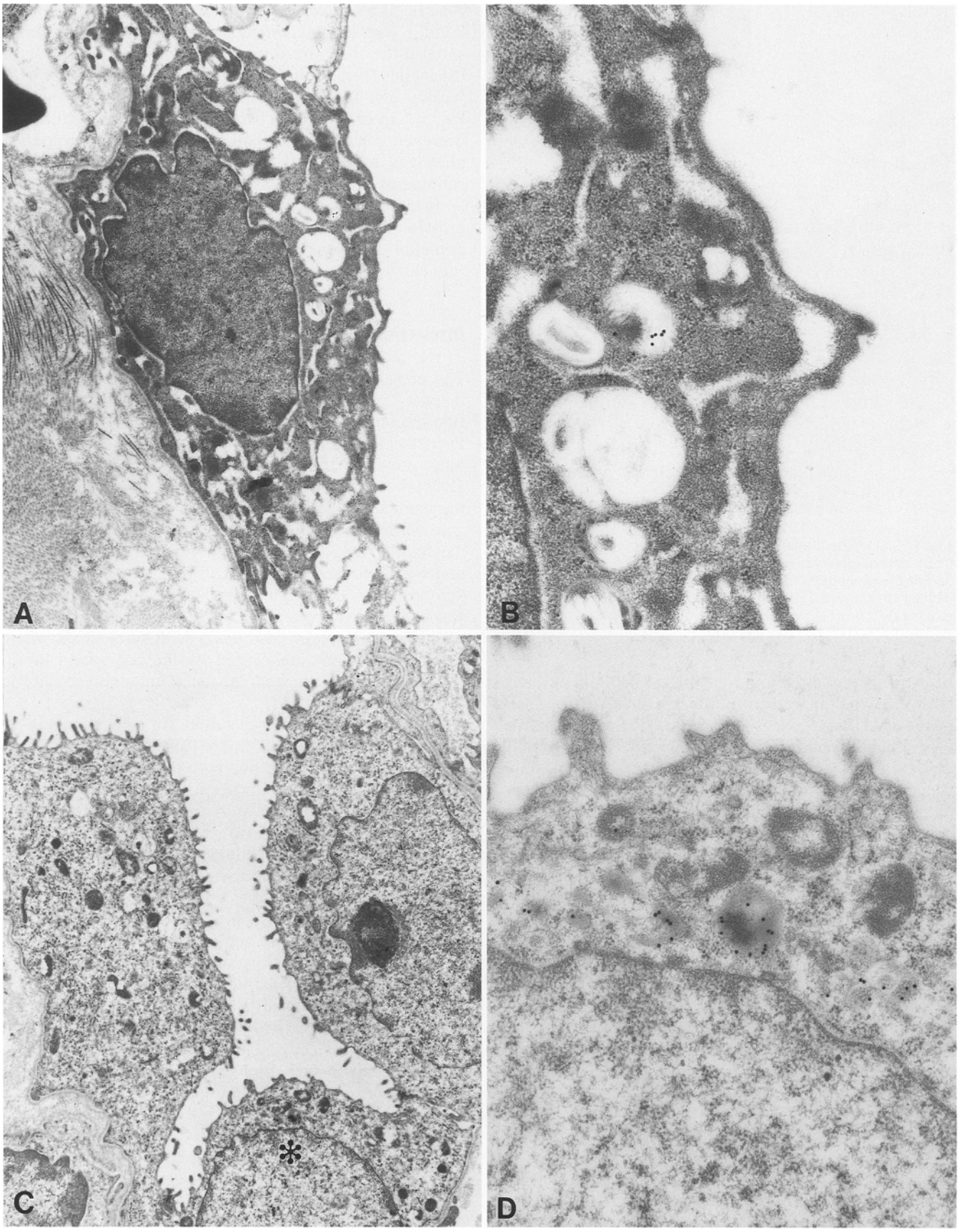

Fig. 8A–D Immunogold localization of cathepsin D in a lysosome of a type II pneumocyte from a relatively normal area (**A, B**) compared with type II pneumocytes present in tubular formations (**C, D**). Note the increased positivity of cathepsin D staining in **C** and **D. B, D** higher magnifications of **A, C** (**D** relates to an area of **C** indicated by an *asterisk*). **A** ×10,500; **C** ×7050; **B, D** ×31,500

broblasts and in the regenerating alveolar epithelium [12].

As far as we know, the localization of any type of proteinases in the alveolar type I cells has not previously been reported. Most of the enzymes investigated in situ in the alveolar epithelium are expressed by alveolar type II cells predominantly (reviewed [12]). The only data for a focal alveolar epithelial expression of cathepsin D are given by Reid et al. [19]. The detection of cathepsin D in normal and injured alveolar epithelium of human lung suggest a biological role for cathepsin D in alveolar epithelial repair and homeostasis. In this context, Morioka and Terayama [18] have shown that cathepsin D specifically stimulates DNA synthesis and mitosis in mouse liver cells and that this proteinase may be somehow involved in the process of cell proliferation. In breast cancer cells its expression is strongly associated with a high proliferation rate [5, 11]. In the present study cathepsin D was found preferentially in proliferative areas of the alveolar epithelium. However, further double staining experiments using proliferation markers including a greater number of samples are needed to confirm this. The cathepsin D gene which is located on the chromosome 11p15 belongs to the oestrogen-regulated genes [2, 17; for review see 20]. Among the 12 patients there were 3 females in which there was no staining of the alveolar epithelium suggesting that sex-dependency can be excluded.

Acknowledgements This work was supported by Bundesminister für Bildung, Wissenschaft, Forschung und Technologie (01ZZ5904). The authors thank Silke Langer and Ingrid Peterson for expert technical assistance.

References

1. Arbustini E, Morbini P, Diegoli M, Grasso M, Fasani R, Vitulo P, Fiocca R, Cremaschi P, Volpato G, Martinelli L, et al (1994) Coexpression of aspartic proteinases and human leukocyte antigen-DR in human transplanted lung. Am J Pathol 145: 310–321
2. Augereau P, Miralles F, Cavailles V, Gaudelet C, Parker M, Rochefort H (1994) Characterization of the proximal estrogen-responsive element of human cathepsin D gene. Mol Endocrinol 8:693–703
3. Bosi F, Silini E, Luisetti M, Romano AM, Prati U, Silvestri M, Tinelli C, Samloff IM, Fiocca R (1993) Aspartic proteinases in normal lung and interstitial pulmonary diseases. Am J Respir Cell Mol Biol 8:626–632
4. Cardozo C, Kurtz C, Lesser M (1992) Degradation of rat lung collagens by cathepsin B. J Lab Clin Med 119:169–175
5. Charpin C, Devictor B, Bonnier P, Andrac L, Lavaut MN, Allasia C, Piana L (1993) Cathepsin D immunocytochemical assays in breast carcinomas: image analysis and correlation to prognostic factors. J Pathol 170:463–470
6. Dobbs LG, Williams MC, Brandt AE (1985) Changes in biochemical characteristics and pattern of lectin binding of alveolar type II cells with time in culture. Biochim Biophys Acta 846:155–166
7. Griffiths G, Simons K, Warren G, Tokuyasu KT (1983) Immunoelectron microscopy using thin frozen sections: application to studies of the intracellular transport of semliki forest virus spike glycoproteins. Methods Enzymol 96:466–469
8. Hernandez-Cueto C, Lorente JA, Pedal I, Villanueva E, Zimmer G, Girela E, Miltner E (1993) Cathepsin D as a vitality marker in human skin wounds. Int J Legal Med 106:145–147
9. Inoue T, Ishida T, Sugio K, Sugimachi K (1994) Cathepsin B expression and laminin degradation as factors influencing prognosis of surgically treated patients with lung adenocarcinoma. Cancer Res 54:6133–6136
10. Ishii Y, Hashizume Y, Watanabe T, Waguri S, Sato N, Yamamoto M, Hasegawa S, Kominami E, Uchiyama J (1991) Cysteine proteinases in bronchoalveolar epithelial cells and lavage fluid of rat lung. J Histochem Cytochem 39:461–468
11. Joensuu H, Toikkanen S, Isola J (1995) Stromal cell cathepsin D expression and long-term survival in breast cancer. Br J Cancer 71:155–159
12. Kasper M, Haroske G (1996) Alterations of the alveolar epithelium leading to pulmonary fibrosis. Histol Histopathol 11:463–483
13. Kasper M, Fuller SD, Schuh D, Muller M (1994) Immunohistological detection of the beta subunit of prolyl 4-hydroxylase inrat and mini pig lungs with radiation-induced pulmonary fibrosis. Virchows Arch 425:513–519
14. Kasper M, Huber O, Großmann H, Rudolf R, Tränkner C, Müller M (1995) Immunocytochemical distribution of E-cadherin in normal and injured lung tissue of the rat. Histochem Cell Biol 104:383–390
15. Kasper M, Günthert U, Dall P, Kayser K, Haroske G, Schuh D, Müller M (1995) Distinct patterns of CD44 isoforms during human lung development and after injury in pulmonary fibrosis. Am J Respir Cell Mol Biol 13:648–656
16. Leto G, Gebbia N, Rausa L, Tumminello FM (1992) Cathepsin D in the malignant progression of neoplastic diseases (Review). Anticancer Res 12:235–240
17. May FE, Smith DJ, Whestley BR (1993) The human cathepsin D-encoding gene is transcribed from an estrogen-related and a constitutive start point. Gene 134:277–282
18. Morioka M, Terayama H (1984) Cathepsin D stimulates DNA synthesis and mitosis in mouse liver in vivo. Exp Cell Res 151:273–276
19. Reid WA, Valler MJ, Kay J (1986) Immunolocalization of cathepsin D in normal and neoplastic human tissues. J Clin Pathol 39:1323–1330
20. Rochefort H (1994) Oestrogens, proteases and breast cancer. From cell lines to clinical applications. Eur J Cancer 30A:1583–1586
21. Ryvnyak VV, Gudumak VSS, Onya ES (1990) Intracellular cathepsin D activity in the liver during cirrhosis and involution. Bull Exp Biol Med Eng Tr 109:250–253
22. Solovyeva NI, Balayevskaya TO, Dialkyan EA, Zakamaldina-Zama TA, Pozdnev VF, Topol LZ, Kisseljov FL (1995) Proteolytic enzymes at various stages of oncogenic transformation of rat fibroblasts. I. Aspartyl and cysteinase proteinases. Int J Cancer 60:495–500
23. Spiess E, Bruning A, Gack S, Ulbricht B, Spring H, Trefz G, Ebert W (1994) Cathepsin B activity in human lung tumor cell lines: ultrastructural localization, pH sensitivity, and inhibitor status at the cellular level. J Histochem Cytochem 42:917–929
24. Tokuyasu KT (1986) Application of cryoultramicrotomy to immunocytochemistry. J Microsc 143:139–149
25. Tokuyasu KT (1989) Use of poly (vinylpyrrolidone) and poly (vinyl alcohol) for cryoultramicrotomy. Histochem J 21:163–171
26. Werle B, Ebert W, Klein W, Spiess E (1994) Cathepsin B in tumors, normal tissue and isolated cells from the human lung. Anticancer Res 14:1169–1176
27. Wildenthal K (1978) Lysosomal alterations in ischemic myocardium. Result or cause of myocellular damage? J Mol Cell Cardiol 10:595–603