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Invited Review

Epithelial lung cell marker: current tools for cell typing

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Summary. This review discusses current immunohistochemical and lectin histochemical approaches to identify and to distinguish the different epithelial cell populations of the pulmonary tissue. Special emphasis is given to the characterization of pulmonary alveolar and bronchial epithelial cells and mesothelial cells. Structural proteins, membrane molecules and secretory products of the alveolar epithelium, which have already been characterized and which may be useful for monitoring the developmental or pathological processes, are listed and briefly described.

Key words: Pulmonary epithelial cells, Antibodies, Immunohistochemistry, Pulmonary fibrosis, Lectins

1. Introduction

The characterization of normal lung morphology is a prerequisite for the study of the respiratory tract under conditions of injury and disease. Besides the electron microscopy for the ultrastructural evaluation of about 40 different pulmonary cell types (Dormans, 1983) the characterization of lung cells has been dramatically improved by the diverse techniques of immunohistochemistry (Funkhouser and Peterson, 1989). The so-called «immunotargeting» can distinguish among morphologically very similar cells during embryonic development as well as among highly dedifferentiated cells in the process of remodelling in case of severe lung injury (e.g. fibrotic and malignant processes). The advent of the monoclonal antibody technology and the improved preparation of diverse secondary conjugates for ABC- and APAAP-techniques as well as for immunofluorescence methods greatly contributed to the tremendous progress in the immunocytochemical typing of cells. As another approach, lectins were introduced to characterize subpopulations of cells. The lectins are carbohydrate binding proteins with defined sugar specificity which bind to the cell surface or cytoplasmic constituents. These approaches supplement the classical staining techniques for selective cell identification; for example, the phosphine 3R fluorescence method (Mason and Williams, 1976) or the NBT-technique for identification of Clara cells (Devereux and Fouts, 1980). The aim of this paper is to present a discussion of the current knowledge of epithelial lung cell markers and their validity for monitoring pathological alterations of lung parenchyma.

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2. Epithelial cell types of the lung

Lung parenchyma contains about 40 different cell types. The epithelial cells lining the broncho-pulmonary system consist of ciliated cells, mucous cells of the surface epithelium and submucosal glands, serous cells of the submucosal glands, basal cells, Clara cells, cells of the neuroepithelial bodies and single neuroendocrine cells, and type I, II and III pneumocytes of the alveoli. In addition, there are mesothelial cells lining the visceral and parietal pleura. These cells can be distinguished on the basis of location, morphology and the various markers discussed below.

3. Cell markers

Cytokeratins

Cytokeratins (CKs), intermediate filament proteins (IF), form an intracellular network of 10 nm filaments and are the characteristic IF proteins of epithelial cells. 20 individual CK polypeptides constitute the cytokeratin family. The two subtypes of CKs are: type I acidic CKs (low molecular weight, 40-56.5 kD) and type II basic-neutral CKs (high molecular weight, 53-67 kD). The CK filaments are heteropolymers and are made of type I and type II chains. The specific polypeptide composition of CKs varies from one cell type to another and only certain differentiation-specific combinations of type I and II CKs occur in vivo, whether in tissues or in cell cultures (Moll et al., 1982; Moll, 1987). Alveolar epithelial cells are characterized by the expression of

four different «simple epithelium type» CKs nos. 7, 8, 18 and 19 according to the Moll catalogue (Moll et al., 1982; Blobel et al., 1984; Table 1). Our own immunolocalization data have revealed a very restricted tissue pattern of expression of CKs in the rat alveolar epithelium: CKs 18 and 8 are the major CKs of type II cells, whereas CK 7 and 19 are predominant CKs of type I pneumocytes (Kasper et al., 1993a). Single CK 18 epitopes are type II cell markers in the rat and the mini pig lung (Kasper et al., 1993a,d). A strong CK19 expression in type II pneumocytes of the rat was found by Paine et al. (1988). The rat type III (=alveolar brush cell) pneumocytes are characterized by abundant CK content with globular expression pattern (Fig. 1), which contrasts with the weaker and more peripheral CK deposition in type II pneumocytes.

The bronchial epithelial cells of the rat exhibit a uniform staining reaction for CK 8, 18 and 19 but a focal staining for CK7 in large bronchi (Kasper et al., 1993a). Species differences, however, have to be taken into account. In human tissues, a uniform CK7 expression in the bronchial epithelial cells, except the basal cells, however, selective staining of basal cells for CK14 and focal CK13 reactions were found (Broers et al., 1989).

Other cytoskeletal elements

In a recent study (Höfer and Drenckhahn, 1992) the actin cross-linking proteins villin and fimbrin were detected in the apical microvilli of rat alveolar and bronchiolar brush cells. This allowed a distinction of type III pneumocytes from the surrounding epithelial cells. Double staining with CK 18-specific antibody Ks18.04 confirmed the identity with the villin-fimbrin-positive brush cell population (Kasper et al., 1994a).

Junctional proteins and cell adhesion molecules

Among the variety of specialized intercellular junctions, those of the adherens type have the most obvious importance for the epithelial cells. The desmosomes are characteristic adhesive junctions of the pulmonary epithelium too. They contain the proteins plakoglobulin and desmoplakin, which are like the desmoglein, a member of the cadherin family (see below), important constituents of desmosomes. Using antibodies specific for the desmosomal type of junctional proteins, a membranous staining of alveolar and bronchial epithelial cells is detectable (Kasper et al., 1993c). Cell adhesion molecules of importance for epithelial cells are members of the integrin family or the cadherins (for review see Takeichi, 1991). Cadherins are Ca^{2+}-dependent cell-cell adhesion molecules that are essential for the formation of physical cell-cell association. The intracellular domain of cadherins interacts with the cytoskeleton. Diverse subclasses have been characterized: E-cadherin (epithelial cadherin); P-cadherin (placental cadherin); N-cadherin (neural cadherin); and L-cadherin (liver cadherin). E-cadherin is detectable in pulmonary epithelial cells (Kasper, unpublished data; Pilewski and Albelda, 1993).

The intercellular adhesion molecule ICAM-1, a member of immunoglobulin supergene family and involved in migration and activation of T-cells and macrophages, is present on type I alveolar epithelial cells (Christensen et al., 1993; Kang et al., 1993).

Lectins

Lectins are defined as a group of carbohydrate-binding proteins. They have a selective capacity to bind polysaccharides or glycoproteins (Goldstein, 1980; Alroy et al., 1984) and they have been used to reveal diverse oligosaccharide profiles of tissues and, more specifically, of single cells (Gelberg et al., 1992). A lot of factors, however, influence the carbohydrate phenotype of a given cell type such as the variability of glycoconjugates among species, among cell types in an individual, between cells in some morphologically uniform cell populations and in cells with different maturation state or under pathological conditions (Spicer and Schulte, 1992). The present review concerns the basic composition of cell surface moieties on alveolar and bronchial epithelial cells of the respiratory tract and some aspects of changing lectin binding pattern under pathological conditions (for a more detailed discussion of the carbohydrate composition of the tracheobronchial epithelium in various species see Spicer et al., 1983; Schulte and Spicer, 1985; Randell et al., 1993).

In the rat pulmonary epithelium, the lectin *Maclura pomifera* agglutinin (MPA) binds to alpha-galactose residues of glycoproteins on the apical surface of type II pneumocytes and displays reactivity with alveolar macrophages (Dobbs et al., 1985; Kresch et al., 1991) and can, therefore, be used as a cytological marker for these cells in the alveoli. The selective binding of MPA to type II cells may relate to a general difference in terminal glycosylation of glycoconjugates on the surface of type I and type II cells (Marshall et al., 1988). The specific binding of MPA was also confirmed by the same authors for the rabbit and man. In mini pig lung we demonstrated the selective labelling of type II cells by using MPA and of type I cells by using *Lycopersicon esculentum* lectin (Kasper et al., 1993c). The MPA-binding protein is an integral membrane glycoprotein with hydrophilic properties (rabbit MPA-gp 230 or rat MPA-gp 200; Marshall et al., 1988). By isolating the MPA-binding glycoprotein, Kresch et al. (1991) identified a 170 kD MPA-binding glycoprotein. Weller and Karnovsky (1989) found an MPA-binding protein (185 kD) in freshly isolated type II cells that is lost during culture. Apical MPA staining is also demonstrable in bronchial epithelial cells, particularly in Clara cells (own unpublished observation on human and rat specimens; Kasper et al., 1993d).

In a recent study by Taatjes et al. (1990) using post-embedding TEM, *Helix pomatia* lectin and *Sambucus nigra* lectin were proposed to be useful markers for rat

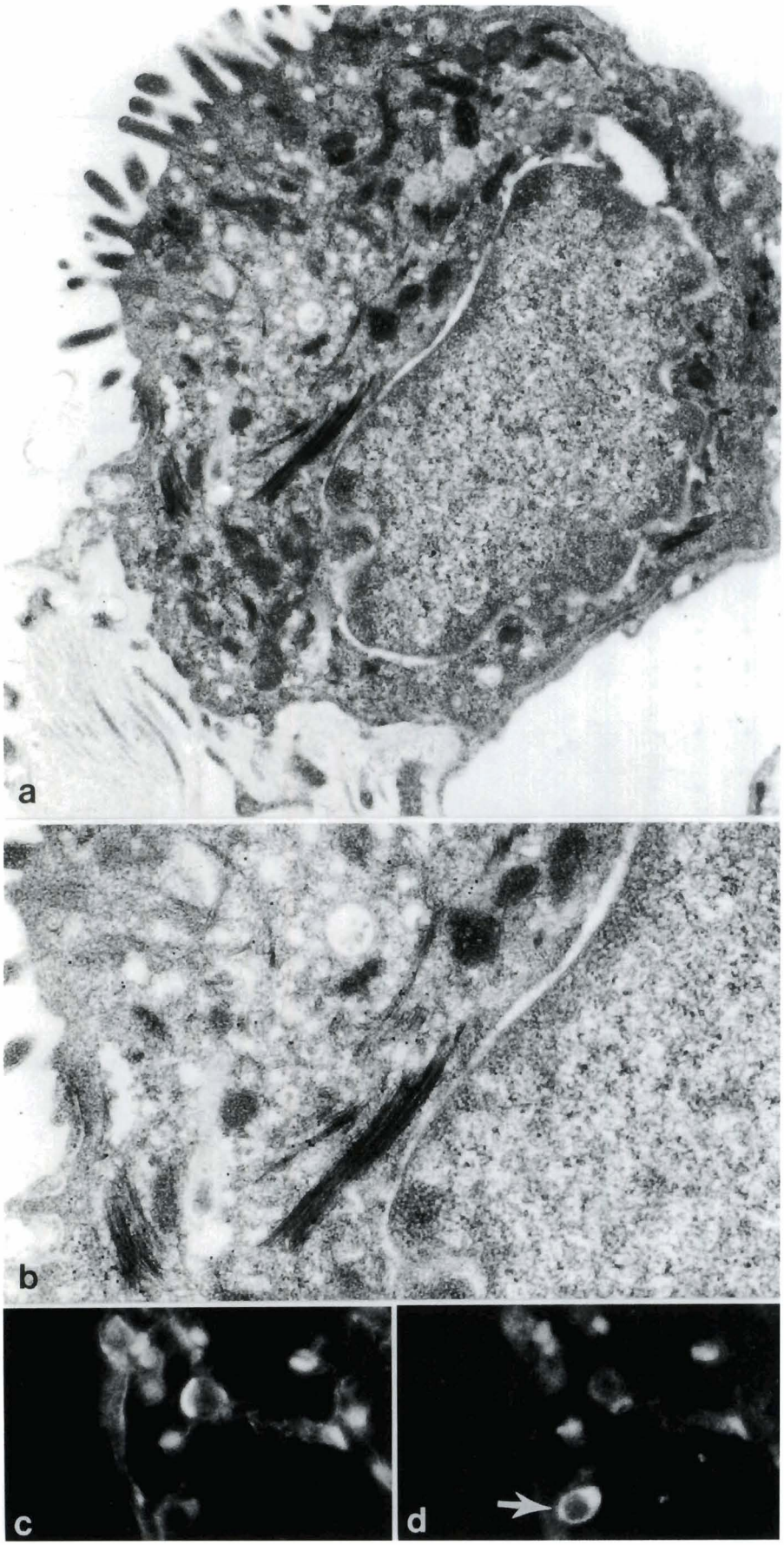

Fig. 1. Rat lung. LR Gold-embedded tissue **(a-b).** Paraffin sections of Schaffer-fixed lung sample **(c-d).** Ultrastructural distribution of cytokeratin 18 (a; b higher magnification of a) in alveolar brush cells using monoclonal antibody Ks18.04 and 10 nm gold-coupled goat anti mouse Ig. Note immunolabelled intermediate filament bundles in b. c-d. Double label immunofluorescence employing c type II cell marker *Maclura pomifera* agglutinin (MPA, FITC-labelled) and d antibody Ks18.04 detected with Texas Red-coupled goat anti mouse Ig. Note the globular cytokeratin pattern of type III cell (arrow), which is MPA-negative. a, x 18,600; b, x 32,250; c-d, x 300

Table 1. Expression profiles of vimentin and cytokeratins in normal lung tissues of different species.

DEVELOPMENTAL STAGE/CELL CULTURE SPECIES/EPITHELIAL CELL TYPE	SIMPLE EPITHELIUM TYPE CYTOKERATINS				SQUAMOUS TYPE TYPE CYTOKERATINS				VIMENTIN	REFERENCE
	8	18	19	7	5, 14, 17	6, 16	13	4		
Foetal development										
Human										
Pseudoglandular period	+	+	+	±						Broers et al., 1989
Canalicular period										
Basal cells	+	+	+	±					±	
Columnar cells	+	+	+	±					±	
Alveolar period										
Basal cells	+	+	+	±	+				±	
Columnar cells	+	+	+	±					±	
Alveolar epithelium	+	+	+	+			±	±		
Monkey										
Pseudoglandular peiod	+	+				+	+			Plopper et al., 1992
Rat										
Pseudoglandular period										Woodcock-Mitchell et al., 1990
Mouse										
Last day of gestation										
Bronchial epithelium	+	+		+						Oomen et al., 1990
Alveolar epithelium				+						
Adults										
Human										
Alveolar epithelium	+	+	+	+	-		-	±		Broers et al., 1989
Bronchial epithelium										
Basal cells	±	±	+	-	±		-	±	±	
Columnar cells	+	+	+	+			±	±	±	
Human										
Alveolar epithelium	+	+								Blobel et al., 1984
Bronchial epithelium										
Basal cells	+	-								
Columnar cells	+	+	+	+						
Human										
Alveolar epithelium	+	+		+	-					Oomen et al., 1990
Bronchial epithelium	+	+		+	±					
Monkey										
Alveolar epithelium	+	+			+	+	+			Plopper et al., 1992
Bronchial epithelium	+	+			+	+	+			
Basal cells	-	-			+					
Type II pneumocytes										
Rat										
Type I pneumocytes			-							Kalina et al., 1993
Type II pneumocytes			+							
Rat										
Type I pneumocytes		+	-							Woodcok-Mitchell et al., 1990
Type II pneumocytes		(+)	+							Mitchell et al., 1991
Rat										
Type I pneumocytes	+	-	+	+						Kasper et al., 1993a
Type II pneumocytes	+	+	(+)	(+)						
Type III cells		+*		+						
Mini pig										
Type I pneumocytes			+	+						Kasper et al., 1993b
Type II pneumocytes		+								
Bronchial epithelium	+	+	+	+					±	
cell culture										
Rat										
Type II cell										
after 1d			(+)							Woodcok-Mitchell et al., 1986
after 3d			+							
after 7d			+							
Rat										
Type II cells										
foetal cells	+	+	(+)	+						Paine et al., 1988
after 21d	+	+	++	+						
adult cells	+	+	+	+						
after 7d	(+)	++	(+)	(+)						

*: globular pattern; +: positive; ±: focal positive; (+): weakly positive; -:negative.

Table 2. For cell typing relevant lectin binding in the rat respiratory tract*.

LECTIN	BINDING PREFERENCE	MAIN PULMONARY REACTVITY	OTHER BINDING	REFERENCE
N-acetylglucosamine group				
Lycopersicon esculentum agglutinin (LEA)	GlcNAc	Type I cells	Cilia of BE	Bankston et a., 1991
N-acetylgalactosamine				
Helix pomatia agglutinin (HPA)	GalNAc-α-1,3GalNAc> α-GalNAc	AM, type II cells	Endothelium	Taatjes et al., 1990
Glycine max agglutinin (SBA)	D-GalNAc	AM	Type I cells**, Single cells of the BE	Schulte and Spicer 1986, Krugluger, 1989 Kasper et al., 1994b
Bauhinia purpurea agglutinin (BPA)	D-GalNAc	Granulocytes Type I cells	Macrophages	Kasper et al., 1994c
Griffonia simplicifolia agglutinin (GS-I-A4)	α-D-GalNAc	AM Basal cells of the BE	Endothelium	Bankston et al., 1991, Shimizu et al., 1991, Simon et al., 1986
Dolichos biflorus agglutinin (DBA)	α-D-GalNAc, D-Gal	AM, Single cells of the BE	AM, Single cells of the BE	Kasper et al., 1993b
Vicia villosa agglutinin(VVA)	α-D-GalNAc	AM		Kasper, unpublished data
Maclura pomifera agglutinin (MPA)	α-D-GalNAc>αGal	Type II cells	AM, Clara cells	Dobbs et al., 1985 Shimizu et al., 1991
Glucose/mannose group				
Pisum sativum agglutinin (PSA)	α-Man>αGlc=GalNAc	Type II cells	Histiocytes	Schulte and Spicer, 1986
Galactose group				
Ricinus communis agglutinin (RCA)	ß-Gal>α-Gal>>GlcNAc	Type I and II cells	AM	Taatjes et al., 1990 Tsukuda and Spicer, 1988
Erythrina cristagalli agglutinin (ECA)	ß-Gal, GlcNAc> GalNAc>Gal	Type I cells		Taatjes et al., 1990
Sambucus nigra agglutinin (SNA)	Gal, GalNAc	Type II cells	Endothelium	Taatjes et al., 1990
Peanut agglutinin (PNA)	Galß1,3GalNAc	AM	Endothelium	Schulte and Spicer, 1986
L-fucose group				
Ulex europaeus agglutinin (UEA)	α-L-Fuc	Endothelium		
Sialic acid group				
Limax flavus agglutinin (LFA)	α-Neu5Ac	Type I and II cells	AM, Endothelium	Schulte and Spicer, 1986

*: excluding the trachea (for review see Spicer et al., 1983; Shimizu et al., 1991); **: after microwave treatment (Kasper, unpublished data); AM: alveolar macrophage; BE: bronchial epithelium.

type II alveolar epithelial cells, whereas *Erythrina cristagalli* lectin selectively stained alveolar type I cells. The *Lycopersicon esculentum* lectin could be successfully used to stain type I pneumocytes of rat (Bankston et al., 1991), mini pig (Kasper et al., 1993c) and human (Kasper, unpublished data). Table 2 demonstrates the diversity of sugar specificity of rat type I cell specific lectins. Selective labelling of type I cells with SBA has been shown in mini pigs (Fig. 2; Kasper et al., 1994c) and rats (Schulte and Spicer, 1986).

Finally, *Bauhinia purpurea* lectin is a further candidate for selective type I cell decoration in rats (Kasper et al., 1994c).

Taken together, lectins are useful probes to detect different epithelial lung cell populations in normal tissues. The rules of cell type-specific expression of glycoconjugates are restricted to the normal adult state of tissue. After pathological alteration, for example in epithelial remodelling during fibrogenesis, the characteristic pattern of lectin binding is no longer maintained. In pulmonary fibrosis, MPA-binding can be lost in proliferating type II pneumocyte clusters (Kasper et al., 1993d). In addition new glycoconjugates are detectable on epithelial cells as seen with *Dolichos biflorus* lectin (Fig. 3a,b).

As far as we know, selective lectin binding to single epithelial cells of the lung parenchyma such as the neuroepithelial cells or the alveolar brush cells has not yet been reported. Characteristic lectin binding pattern of the bronchial epithelial cells have so far not been fully evaluated (Table 2). Furthermore, we have to take into account the changes in glycoconjugate composition in the developing airways suggesting a special differentiation-dependent mode of lectin

binding pattern (Ito et al., 1990). These alterations in the expression of sugars diminish the value of lectins for monitoring cells during development. Nevertheless, lectin binding pattern reflects the modulation of carbohydrate composition on the surface or in the cytoplasm of cells, which is associated with cellular differentiation and functional maturation (Schulte et al., 1990).

Secretory proteins

Mucus: Mucus is secreted by the submucosal bronchial glands as well as the goblet cells in surface epithelium of bronchi. Mucus secreting cells are generally absent from the alveoli. A number of different types of mucins are secreted by the serous and mucinous types of glands. Antibodies to mucous proteins can be

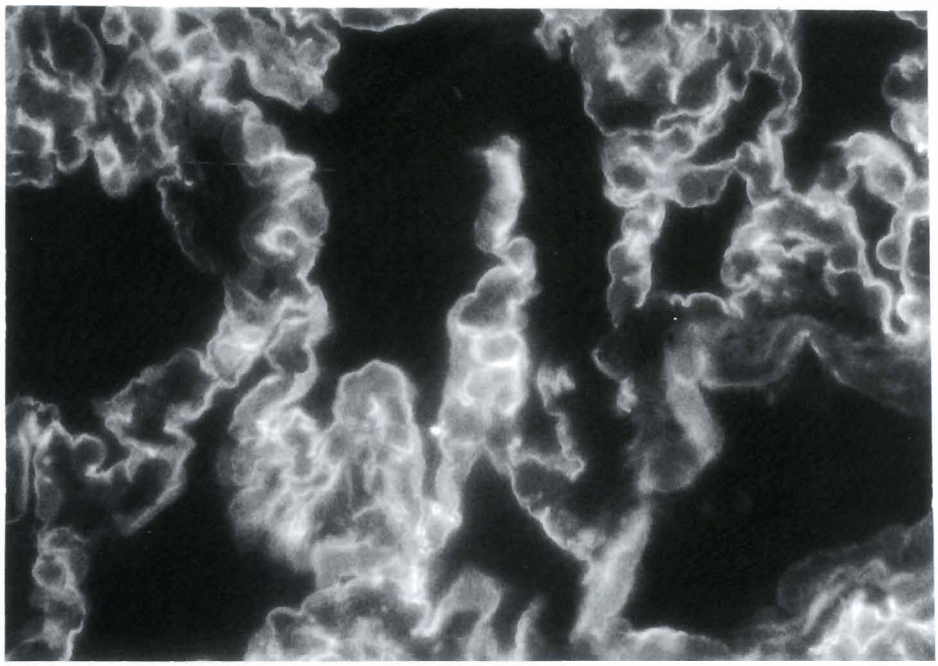

Fig. 2. Mini pig lung. Paraffin section. Selective binding of Soybean agglutinin to type I alveolar epithelial cells. Immunofluorescence x 550

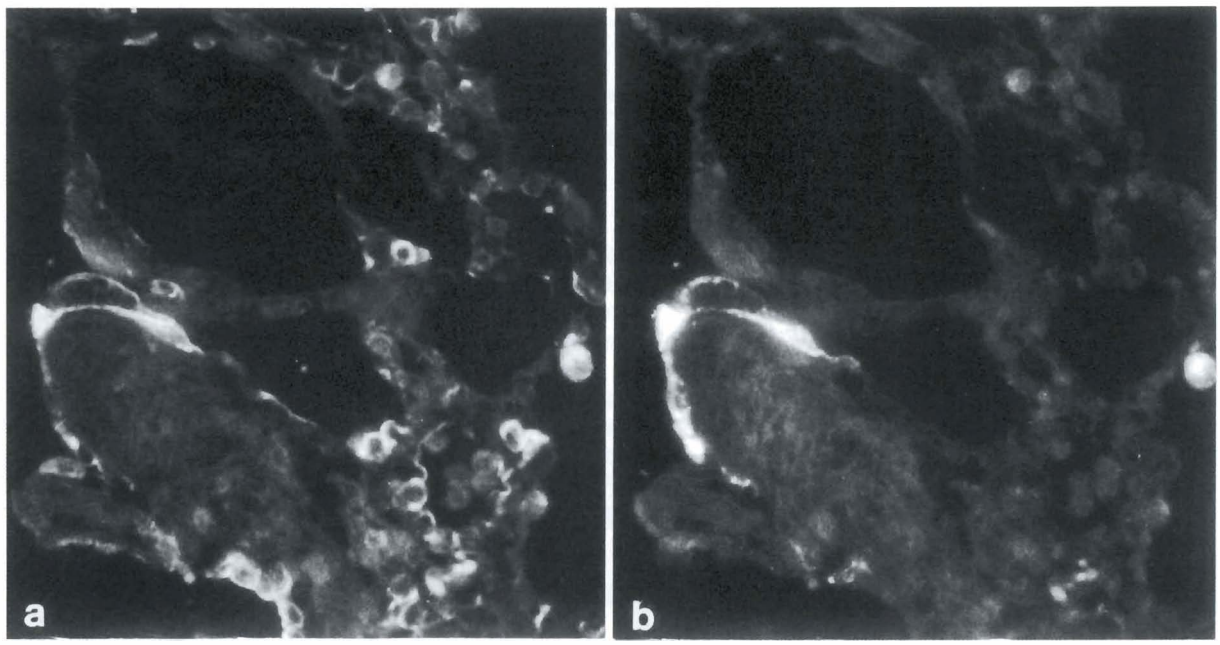

Fig. 3. Rat lung after bleomycin installation. Paraffin section. Double label immunofluorescence with polyclonal anti cytokeratin antiserum **(a)** and Dolichos biflorus agglutinin (DBA; **b**). DBA binding is usually restricted to alveolar macrophages (Kasper et al. 1993b). In reepithelialized areas, focal DBA binding can be detected with epithelial cells (b). a-b x 300

used as markers for mucus secreting cells (StGeorge et al., 1985). Lysozyme (as described below) can be used as a reliable marker for serous sub-mucosal glandular cells.

Lysozyme: Lysozyme, a low molecular weight basic protein with antibacterial activity, is secreted by a number of mucosal cells and glands. In the tracheo-bronchial tree of human lung lysozyme is present in the serous submucosal cells. However, in animals lacking sub-mucosal glands, e.g. rat, lysozyme is synthesized and secreted by type II pneumocytes (Singh et al., 1988a,b; Franken et al., 1989). In such animals lysozyme serves as a marker for type II pneumocytes. Intraalveolar macrophages synthesize and stain for lysozyme.

Secretory leukocyte-protease inhibitor: A low molecular weight protease inhibitor with activity against neutrophil elastase is secreted by a number of mucus epithelial cells including submucosal glands in the airways and Clara cells. Antibodies to the protein can be used as markers for mucus secreting cells and Clara cells (DeWater et al., 1986; Franken et al., 1989).

Clara cell 10 kDa protein: Non-ciliated, non-mucus type of secretory cells in the airways of the lung secrete a uteroglobin like low molecular weight protein. Immunohistochemically the protein serves as a specific marker for Clara cells. However, by in situ hybridization mRNA for CC10 can be detected in alveolar cells albeit at much lower levels than that found in Clara cells (Shiratori et al., 1994). The protein can be detected in pathological lesions of the lung, e.g. in ozone-induced changes and in pulmonary neoplasms (Singh and Katyal, 1984; Hegele-Hartung and Beier, 1985; Singh et al., 1985, 1988a,b; Bedetti et al., 1987; Singh and Katyal, 1991, 1992; Masaro et al., 1994).

Beta galactose binding lectin: Antiserum to a beta galactoside binding protein in rat lung specifically stains Clara cells. The protein is present in bronchoalveolar lavage. Its reported size, 29 kDa, is similar to the size of tryptase Clara (Wasano and Yamamoto, 1989).

Surfactant protein A (SP-A): Surfactant protein A is a glycoprotein with variable glycosylation and is secreted predominantly by type II pneumocytes. However, antibodies to surfactant protein A variably stain airway cells, in particular, Clara cells in the distal airways. Message for SP-A can be readily detected in both alveolar as well as airway nonciliated cells. The protein is present in foetal lungs and can usually be detected in the lung in pathological states including neoplasms of the lung in experimental animals as well as humans (Katyal and Singh, 1979; Balis et al., 1985; Coalson et al., 1986; Kuroki et al., 1986; Walker et al., 1986; Phelps and Flores, 1988; Possmayer, 1988; Williams et al., 1988; Hawgood, 1989; Williams and Dobbs, 1990).

Surfactant protein B (SP-B): This hydrophobic protein is present in a similar distribution as described for SP-A, i.e. it can be detected in alveolar type II cells as well as bronchial Clara cells (Weaver et al., 1988; Pilot-Matias et al., 1989).

Surfactant protein C (SP-C): This extremely hydrophobic protein has been difficult to detect by conventional antibodies. However, examination with nucleotide probes for the message for SP-C has shown that the protein is restricted to alveolar type II cells (Wikenheiser et al., 1993).

Surfactant protein D (SP-D): Like SP-A and SP-B, surfactant protein D can be readily detected in both alveolar type II cells as well as bronchial Clara cells (Persson et al., 1989). Very recently, we have generated, a panel of monoclonal anti-bodies againt SP-D. Interestingly, SP-A and SP-D differ in their localization in bronchiolar Clara cells (Fig. 4a,b).

Enzymes

To our knowledge, most available data on the enzymes used as marker proteins of pulmonary epithelial cells concern only type II pneumocytes, which produce alkaline phosphatase, although Clara cells also stained for this enzyme activity (Edelson et al., 1988). In a recent light- and electron microscopical study using enzyme-histochemical and immunohistochemical techniques the heat-stable isoenzyme of alkaline phosphatase in the human and monkey lung was investigated (Nouwen et al., 1990). The enzyme was selectively present in all type I cells and in cuboidal epithelial cells of bronchioli.

Another enzyme, the carbonyl reductase, was employed to label mainly Clara cells and to a lesser extent ciliated cells of the guinea pig and mouse lung (Matsuura et al., 1990).

Cytochrome P-450 is found in Clara cells and type II cells (Boyd, 1977; Devereux et al., 1981) and used as marker for these cells within the lung. Because electrolytes and fluid are actively transported across the alveolar epithelium (cf. Saumon and Basset, 1993) the corresponding Na^+K^+-ATPases are localized in the plasma membrane. Further, Na^+ channel proteins were immunohistochemically colocalized with the MPA lectin on the apical surface of type II pneumocytes (Malaton et al., 1992). Na^+K^+-ATPase is readily detectable, cytochemically, in type II cell, though it is present in lower amounts in type I and endothelial cells (Schneeberger and McCarthy, 1986).

Trypsin-like proteases are involved in various biological processes, particulary in the defencece against viral infection. They are well characterized in pulmonary mast cells. Kido et al. (1992) found a novel protease, named tryptase Clara, in the bronchial epithelial cells of the rat. Carboxypeptidase M, a vasoactive peptide cleaving enzyme, could be localized

to type I alveolar cells (Nagae et al., 1993). Uniform labelling of epithelium was detected with anti-nitric oxide synthase antibodies in both human bronchi and normal rat trachea samples (Kobzik et al., 1993). Selective localization of the ß subunit of prolyl 4-hydroxylase, an enzyme involved in collagen and surfactant protein metabolism, was found in human type II alveolar cells (Kasper et al., 1994d; Fig. 5a,b). In cases of fibrosis enhanced immuno-reactivity of the enzyme in epithelial as well as non-epithelial cells was detectable (Kasper et al., 1994d). For other epithelial cell enzymes see the review of Simon (1992).

Cytokines and components of the coagulation cascade

A number of cytokines are involved in pulmonary disease such as, I1-1, TNF alpha, TGF-ß and PDGF (Piguet, 1993). Interestingly, TNF alpha has been shown to be localized in human type II pneumocytes, weakly in the bronchial epithelium and in alveolar macrophages (Nash et al., 1991). In cases of cryptogenic fibrosing alveolitis, hyperplastic type II cells and areas of alveolar

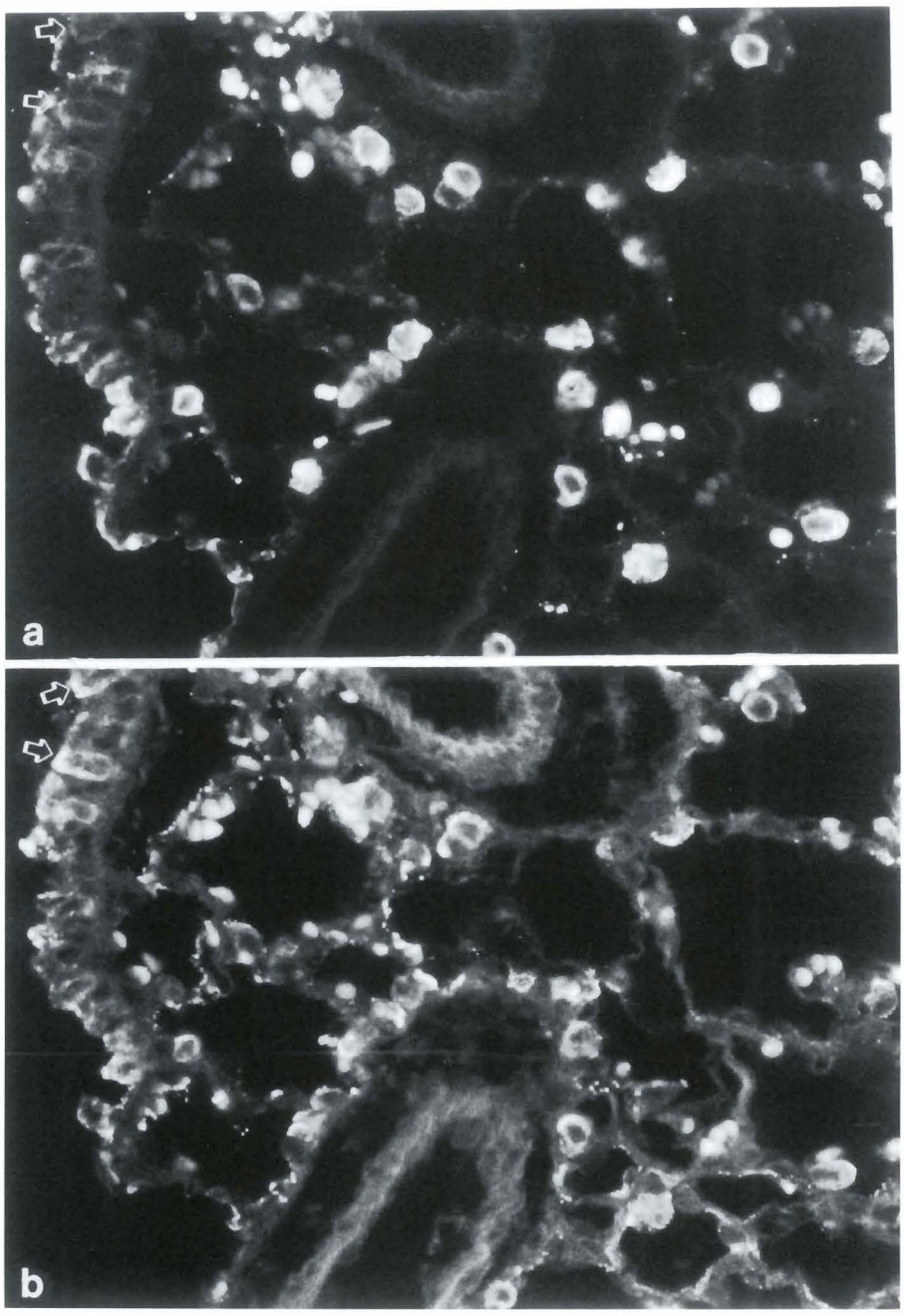

Fig. 4. Normal rat lung. Simultaneous localization of SP-A **(a)** and SP-D **(b)**. Arrows indicate two SP-A$^-$/SP-D$^+$ clara cells. Note the more peripheral MPA-like SP-D staining of type II alveolar epithelial cells. a-b, x 300

bronchiolization showed the most prominent reaction (Nash et al., 1993). Piguet et al. (1993), however, found the TNF alpha protein and the corresponding mRNA in some interstitial cells of the normal lung only, whereas in idiopathic pulmonary fibrosis a strong immunoreaction for TNF alpha as well as elevated mRNA level could be detected in the alveolar epithelium.

Finally, type II pneumocytes express plasminogen activator and tissue factor according to their role as contributors to alveolar fibrin deposition (Marshall et al., 1991). In addition bronchial epithelial cells and focally type I cells were labelled with tissue factor-specific antibodies in human lung specimens (Drake et al., 1989).

Other antigens

A number of other membrane and cytoplasmic antigens have been identified that may be useful in distinguishing epithelial cell types. The first group to be discussed is the Heyman nephritis antigen on the surface of type II cells of the lung (Singh and Makker, 1985). Heyman nephritis is an autoimmune membranous glomerulonephritis experimentally inducible in rats (Kerjaschki and Farquar, 1983). Diverse mannose-

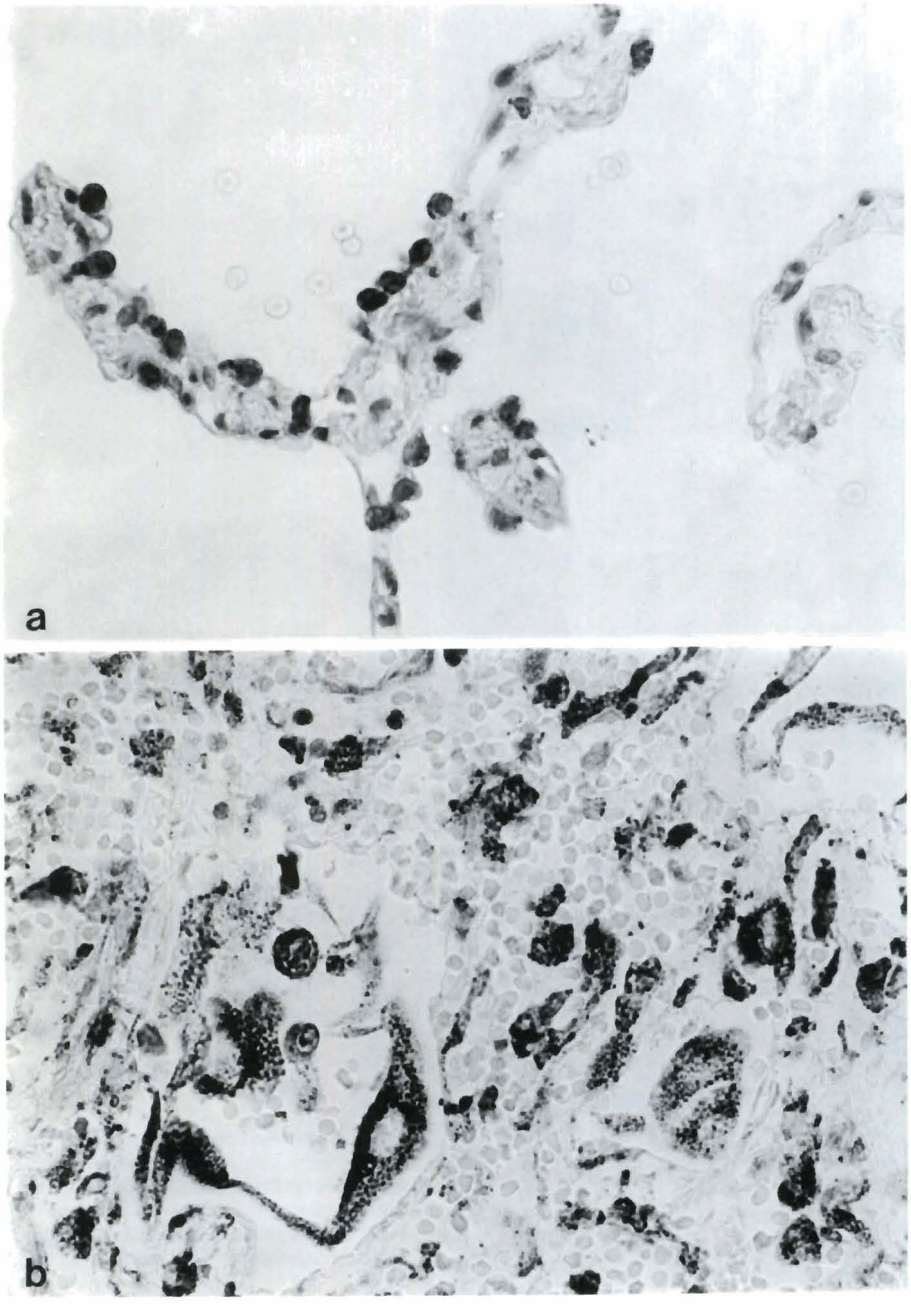

Fig. 5. Human lung. **a)** Normal and **b)** injured tissue (pulmonary fibrosis after therapeutic irradiation). Immunoperoxidase demonstration of the ß subunit of prolyl 4-hydroxylase in type II cells **(a)** and in epithelial cells, in alveolar macrophages and interstitial cells **(b)**. Mayer's haematoxylin counterstaining. a-b, x 350

containing glycoproteins of different molecular weight (gp600, gp330, gp280) were identified at the microvillar bases or in clathrin-coated areas of the proximal renal tubular epithelial cells by using polyclonal and monoclonal antibodies (Sahali et al., 1993). Due to the existence of cross-reacting antigenic determinants in the lung, several authors reported reactive epitopes in lung parenchyma on type II pneumocytes (Makker et al., 1989; Bachinski et al., 1993). By utilizing a polyclonal anti-gp600 antiserum Kotas et al. (1991) found additional expression of the Heyman nephritis antigen on the surface of Clara cells. Another glycoprotein, pneumocin (Mr 165kD), was isolated by MPA lectin affinity chromatography (Lwebuga-Mukasa, 1991). Monoclonal antibodies to pneumocin labelled the apical membrane of mature type II pneumocytes of the rat. Pneumocin, a sialoglycoprotein with N-linked oligosaccharide side chains, is distinct from surfactant apoproteins and also from MPA-binding glycoproteins. The latter have been characterized from several species as proteins ranging from 185kD to 330 kD molecular weight (Marshall et al., 1988; Weller and Karnovsky, 1989). Their expression occurred in association with the apical differentiation process and is hormone-

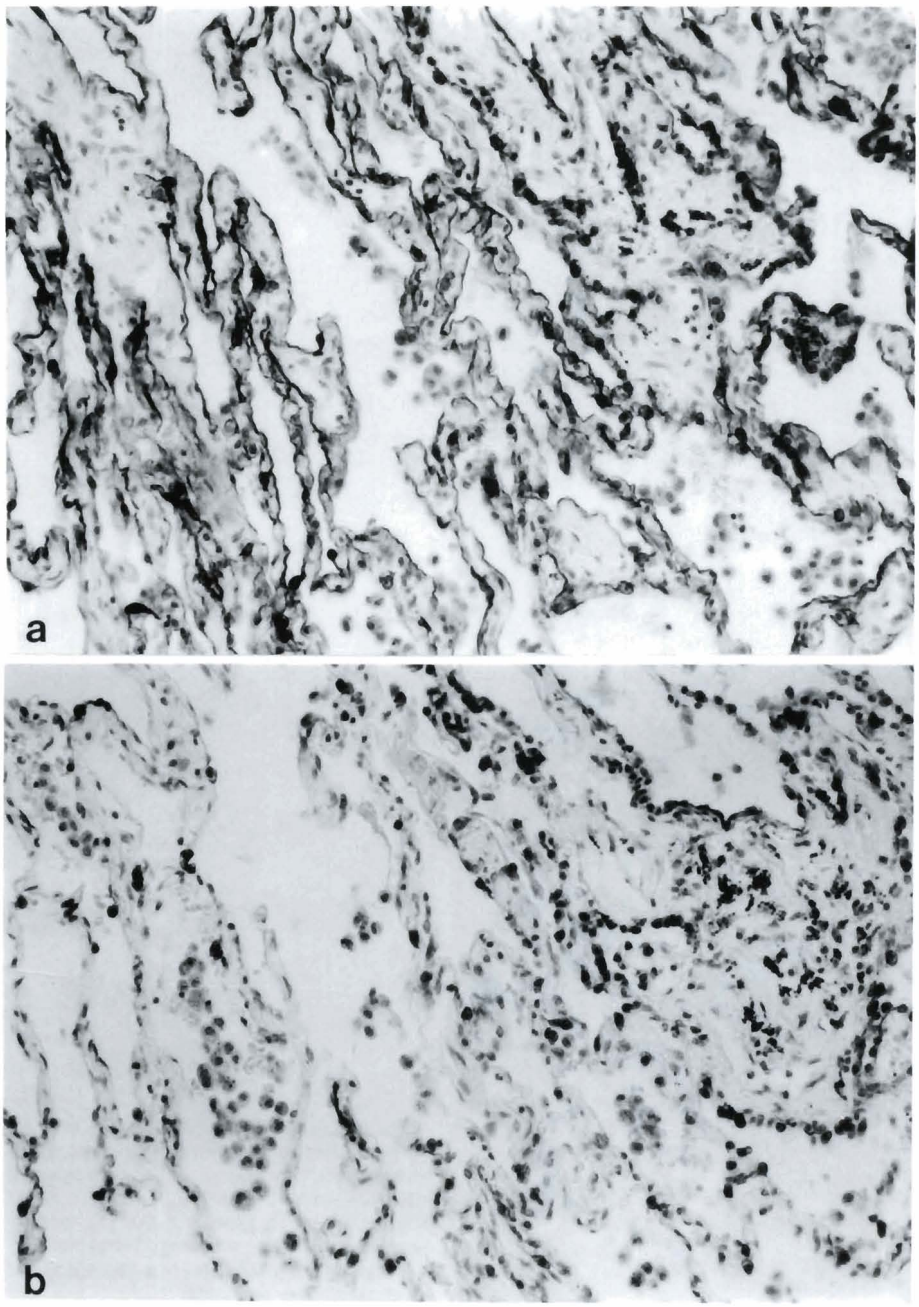

Fig. 6. Serial sections of normal human lung tissue. Demonstration of cytokeratin 7 in type I and type II alveolar epithelial cells (**a**). EMA immunoreactivity (**b**) is restricted to type II pneumocytes ABC technique. Mayer's haematoxylin counterstaining. a-b, x 180

independent (Joyce-Brady and Brody, 1990).

Funkhouser et al. (1987) reported on the p146 antigen (JBR-1 monoclonal antibody) present on the apical surface of type II cells and on the luminal surface of the small bowel epithelium and the proximal kidney tubules. Rat type II pneumocytes and Clara cells were labelled with monoclonal antibodies specific for the cell luminal surface (Miller et al., 1989). In human and monkey lungs, Suehiro et al. (1989) demonstrated a 60kD protein in type II pneumocytes and nonciliated bronchial epithelial cells. The peptide antigen was different from the Ca1 antigen, which is a glycoprotein of a number of carcinomas and selectively bound to human type II pneumocytes (Brody et al., 1988). A marker for both the alveolar epithelial cells as well as the bronchiolar epithelium represents the epithelial membrane antigen (EMA; Hara et al., 1989). EMA is a high molecular weight glycoprotein (256-400kD), isolated from human milk (Ormerod et al., 1983) and regarded as a marker of epithelial and mesothelial differentiation (Pinkus and Curtis, 1985). In our experience, EMA antibodies preferably react with type II pneumocytes and only focally with (pathologically altered?) type I alveolar epithelial cells (Fig. 6a,b).

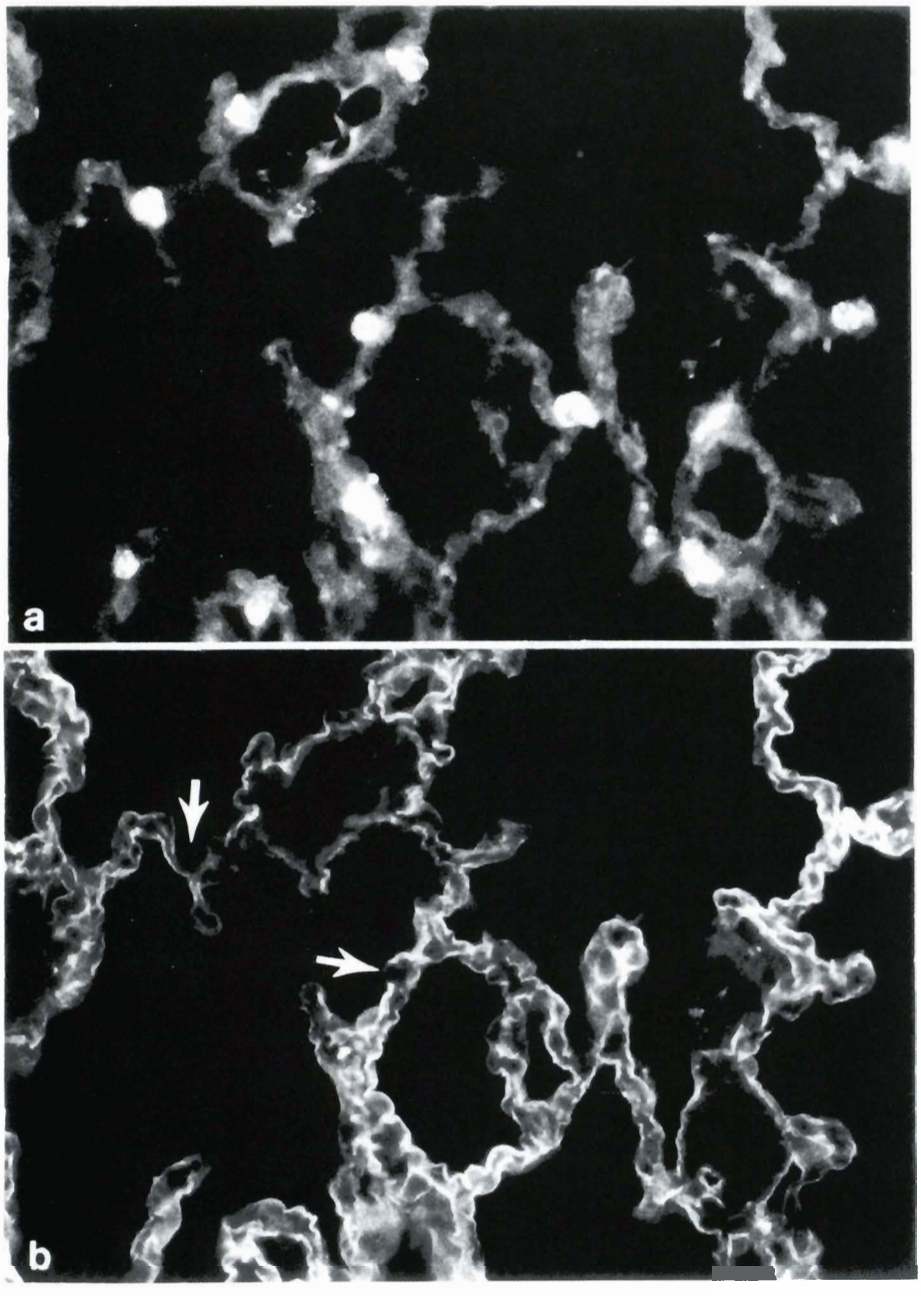

Fig. 7. Normal rat lung. Double label immunofluorescence for the localization of type I cell marker MEP-1 **(b)** and surfactant protein A (SP-A) **(a)**. Note the interruption of epithelial lining at places of SP-A-positive type II cells (arrows). a-b, x 300

Type I pneumocytes represent only 5-8% of lung parenchymal cells, but cover about 90% of the alveolar surface. While type II cells synthesize and secrete surfactant (see above) and are involved in trans-epithelial transport processes, little is known of type I cell function. Consequently, few data exist about the antigenic profile of these cells. Dobbs et al. (1988) at first developed monoclonal antibodies that bound to rat type I cells in situ. Very recently, Hotchkiss et al. (1992) developed a rat monoclonal antibody specific against a membrane-associated epitope of murine type I cells. The exact nature of the antigen in not yet fully characterized, but the antibody recognizes the antigen on mature, terminally differentiated type I cells only. Tsuchiya et al. (1993) generated a monoclonal antibody MEP-1 against rat histiocytoma cells. This antibody shows an additional selective type I specificity (Fig. 7a,b). Further type I cell-specific monoclonal antibodies were described by Danto et al. (1992) and Singh et al. (1993).

Mesothelial cell markers

Markers for cytoplasmic and cell surface molecules for mesothelial cells have been described and can be used to identify these cells in tissue sections. The markers appear to be common to pleural, pericardial and peritoneal lining. Antibodies to cytoplasmic and cell surface marker have been prepared by using either cultured mesothelial cells or mesothelioma cell lines (Singh et al., 1979; Donna et al., 1986; Anderson et al., 1987; Hsu et al., 1988; Stahel et al., 1988; Chang et al., 1992). Some of the antibodies generated by using mesothelioma cells lines as the immunogen react with mesotheliomas but not with normal mesothelial cells. Other antibodies to mesothelial cells cross react with respiratory epithelial cells. We have generated a monoclonal antibody to lung lavage proteins that reacts with ciliated cells and mesothelial cells. The reactivity with ciliated cells is independent of ciliation (unpublished observation).

4. Comments

The various markers for different epithelial cell types in the lung have been addressed for normal lungs. It should be noted that in pathological states, the normal expression of different markers may not apply as has been alluded to in some of the cases. Nevertheless, a knowledge of the distribution of normal cell markers should facilitate a study of development and pathology of the lung. A more detailed investigation of the «markers» resulting in their molecular characterization may reveal their functions and indirectly add to the information about the functions of different cell types.

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