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Modulation of Actin Isoform Expression in Alveolar Myofibroblasts (Contractile Interstitial Cells) During Pulmonary Hypertension

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Lungs of 37 patients with pulmonary hypertension (PHT), 5 normal human lungs, and 30 normal rat lungs, were studied using immunohistochemical stainings for actin, alpha-smooth muscle (alpha-SM) actin and desmin. The type of PHT was determined on clinicopathologic grounds (in 17 cases by catheterism); 20 patients had precapillary and 17 postcapillary PHT. In normal lungs, myofibroblasts, ie, contractile interstitial cells (CIC), distributed in alveolar septa, were not stained by alpha-SM actin antibodies. Only around the venules, were cells labeled by this antibody present. Furthermore, there were bundles of alpha-SM actin-positive cells around the openings of air sacculi into the alveolar ducts. In precapillary PHT, the distribution and immunostaining properties of interstitial cells remained unchanged; alpha-SM actin-positive cells were observed in thickened arterial intima and in plexiform lesions. In postcapillary PHT secondary to heart failure, to mitral stenosis, or in veno-occlusive disease, many interstitial cells in the alveolar septa were decorated by alpha-SM actin antibodies but not with desmin. The authors propose that, in postcapillary PHT, mechanical stretch due to capillary congestion may be responsible for the generation of cells that express an actin isoform associated with smooth muscle. (Am J Pathol 1990, 136:881–889)

Normal alveolar septa contain interstitial cells laden with microfilaments.[1-3] These cells stain with anti-actin antibodies, and they have contractile properties. In hypoxic media as well as when stimulated by epinephrine, fine strips of lung tissue contract, and this contraction has been attributed to that of interstitial cells.[1-3] Because of their functional features, they were designated 'contractile interstitial cells' (CIC) of alveolar septa.[1] In fact, they correspond immunohistochemically and ultrastructurally to myofibroblasts.[4,5]

The existence of these cells in normal alveolar tissue[6-10] as well as in pathologic conditions[10-15] has been confirmed by many investigators. Alveolar myofibroblasts have been shown to proliferate in idiopathic lung fibrosis as well as in bleomycin-induced pulmonary lesions.[9] In early studies the relationship of myofibroblasts (including alveolar myofibroblasts) to smooth muscle cells and to fibroblasts remained obscure.[16]

Recently, it has been proposed that cytoskeletal features of cells, and in particular the presence of alpha-smooth muscle (alpha-SM) actin isoform (one of the six actin isoforms present in higher cells of vertebrates) is a reliable marker of smooth muscle differentiation[17]; a monoclonal antibody has been raised against this isoform.[18] Use of this antibody has shown that stromal cells of several normal organs and of carcinomas, as well as myofibroblasts of different types of fibromatoses, include alpha-SM actin-laden cells.[19-24] These cells have been also called 'myoid' cells.[20] Recently, the presence of numerous alpha-SM actin-containing cells in alveolar septa has been reported in bleomycin-induced pulmonary fibrosis in the rat.[25]

The aim of the present study was to investigate whether modifications in pulmonary hemodynamics may promote the development of alpha-SM actin in alveolar myofibroblasts. For this purpose we have chosen cases of precapillary and postcapillary pulmonary hypertension,

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Table 1. *Material: Distribution of Precapillary (pre-c) and Postcapillary (post-c) Pulmonary Hypertension*

No.	A/S	Age	Sex	Etiology	Type of hypertension	Follow-up (years)	PAP mm Hg
1	A	50	F	RPE	pre-c	<1	100/70
2	A	35	F	Idiopathic	pre-c	2	92/57
3	A	35	F	Aminorex*	pre-c	4	120/75
4	A	68	F	Aminorex	pre-c	9	120/80
5	A	66	F	Aminorex	pre-c	11	110/60
6	A	63	F	Aminorex	pre-c	10	
7	A	66	F	Aminorex	pre-c	15	75/50
8	A	55	F	Aminorex	pre-c	8	
9	S	75	M	Idiopathic	pre-c	4	
10	A	72	F	Idiopathic	pre-c	10	
11	S	73	F	Aminorex	pre-c	15	114/66
12	A	50	M	Idiopathic	pre-c	5	120/70
13	A	80	M	RPE	pre-c	1	
14	A	52	M	RPE	pre-c	3	
15	A	75	F	Aminorex	pre-c	8	100/55
16	S	39	F	Idiopathic	pre-c	5	60/35
17	S	25	M	Idiopathic	pre-c	1	60/42
18	S	35	F	Idiopathic	pre-c	2	110/65
19	S	40	F	Idiopathic	pre-c	3	80/35
20	S	23	M	Idiopathic	pre-c	1	65/30
21	A	83	M	CHF	post-c	1	
22	A	59	F	CHF	post-c	1	
23	S	53	M	MS	post-c	>20	
24	S	21	F	VOD	post-c	<1	73/50
25	A	61	F	MS	post-c	2	
26	A	75	F	CHF	post-c	7	
27	A	66	F	CHF	post-c	1	
28	A	93	F	CHF	post-c	1	
29	S	20	M	VOD	post-c	1	80/42
30	A	17	F	VOD	post-c	<1	
31	A	25	M	MS	post-c	2	
32	A	65	M	VO-Ca	post-c	1	
33	S	30	M	CHF + PF	post-c	2	65/45
34	A	72	F	CHF	post-c	4	
35	A	80	F	CHF	post-c	4	
36	A	71	M	CHF	post-c	8	
37	A	68	M	CHF	post-c	3	

* Aminorex (or Menocil): an anorexigen drug used in central Europe in early 1970s.

A/S = material obtained at autopsy (A) or surgery (S). PAP = pulmonary arterial pressure. RPE = repeated pulmonary embolism. CHF = chronic heart failure. MS = mitral stenosis. VOD = veno-occlusive disease. VO-Ca = venous obstruction by a carcinoma. CHF + PF = chronic heart failure + pulmonary fibrosis.

and compared the distribution of actin and alpha-SM actin antibody-labeled cells with that observed in control lungs. Human lungs devoid of vascular pathology and normal rat lungs were used as controls.

Material and Methods

Thirty-seven human lungs from cases of pulmonary hypertension were investigated; in 17 cases, cardiac catheterism data were available. The age, the sex, the type of the pulmonary hypertension, and the follow-up period of each case are summarized in Table 1. These lungs were compared with five human lungs without pulmonary vascular or cardiac pathology and with 30 normal rat lungs (20 fixed by intratracheal instillation and 10 by vascular perfusion).[26] The lung samples in cases of pulmonary hypertension as well as human controls were obtained at autopsy (26 hypertensions, 3 controls) or from open-lung biopsies (one hypertension, two controls); they were fixed by immersion in 10% formaldehyde. The material was embedded in paraffin, sectioned on a rotating microtome at 5-μ thick serial sections. From these serial sections, the first one was stained with hematoxylin and eosin (H&E), the second with a polyclonal anti-actin antibody,[27] the third with normal rabbit serum (control section), the following section with a monoclonal alpha-smooth muscle (alpha-SM) actin antibody,[18] and the last with mouse gamma G immunoglobulin (IgG) (control section). Both antibodies were prepared in our laboratories, the anti-actin antibody being raised in the rabbit according to the method reported by others.[27] The specificity and sensitivity of both

antibodies were tested and reported elsewhere.[16-18] In some cases, immunostaining with polyclonal affinity purified anti-desmin antibodies (raised in the rabbit in the laboratory of one of us: GG — [19]) also was performed. The immunostaining of sections was performed using Avidin-Biotin Complex (ABC) method.[28]

On the basis of histologic findings and clinical studies, including the hemodynamic data, the pulmonary hypertension cases were divided into precapillary and postcapillary types (Table 1). In the precapillary pulmonary hypertension group, there were nine cases of plexogenic pulmonary hypertension, eight cases of pulmonary hypertension secondary to aminorex intake,[29] and three cases of pulmonary hypertension with microthrombotic lesions.[30]

Postcapillary pulmonary hypertension material included 10 cases of congestive heart failure, three cases of mitral stenosis, three cases of veno-occlusive disease, and one case of pulmonary venous thrombosis secondary to invasion of the pulmonary vein by a bronchial carcinoma.

Results

In control lungs, human as well as in the rat, alveolar septa contained actin-laden interstitial cells. These cells were distributed in a more or less regular pattern and amounted to five to eight cells around each air sacculus (Figure 1a and b); they were more numerous around small arteries and venules and along the alveolar ducts. As expected, vascular and bronchial muscles were intensely stained.

Alpha-smooth muscle actin antibody decorated the vascular walls as well as bronchiolar musculature. Around arterioles and venules, a rim of cells labeled by this antibody was visible (Figure 1c and e). These cells were considered to represent the pericytes. In the alveolar septa, no alpha-SM actin-laden cells were seen (Figure 1c, d, and e). The ring of smooth muscle located at the opening of the alveoli into the alveolar ducts was deeply stained with alpha-SM actin antibody. On cross sections, this ring appeared as 'raquette-shaped' smooth muscle cell bundles (septal tips) situated at each side of the openings of air sacculi into the duct (Figure 1d).

Antibody against desmin stained arterioles and bronchial muscle as well as the bundles of muscle cells located along the alveolar ducts. No staining of cells surrounding the venules (pericytes) was observed. Furthermore, no alveolar interstitial cell was labeled.

In precapillary pulmonary hypertension, alveolar septa were thin, their cellularity was not increased, and capillaries appeared normal. The walls of muscular pulmonary arteries were thickened, and this thickening was due to hypertrophy of the media and to a cellular and/or fibrillar thickening of the intima. The thickened intima appeared predominantly cellular in arterioles and in arteries below 200 μ in diameter. In many cases, some small ramifications of muscular pulmonary arteries contained typical plexiform lesions (Figure 2b). (For more detailed description of arterial changes in precapillary pulmonary hypertension, see Widgren[29]; for those of microthromboembolic syndrome, see Pietra et al[30]).

The distribution of actin- and alpha-SM actin-laden cells in alveolar tissue was the same as in normal lungs; namely, cells labeled by actin antibody were distributed all through the alveolar septa, whereas alpha-SM actin-positive cells were visible only around arterioles and venules, as well as at the opening of the alveoli into the alveolar ducts (Figure 1f and g). Anti-desmin antibodies labeled again the same structures as in normal lungs. Interestingly, the thickened arterial intima of muscular pulmonary arteries contained a large number of elongated cells that were decorated with alpha-SM actin antibody. Many of these cells showed a circular orientation around the lumen. Furthermore, the plexiform lesions appeared to be composed predominantly by alpha-SM actin-positive cells (Figure 2a). In some cases, a noticeable increase in the muscular coat of small arteries and some crowding of alpha-SM actin-positive cells around arterioles occurred.

In cases of pulmonary hypertension due to microthrombotic lesions,[30] the distribution and staining patterns of interstitial cells were similar to that of plexogenic pulmonary hypertension; namely, in alveolar septa there was no increase or change in the distribution of alpha-SM actin-positive myoid cells. However, such cells occurred in variable number in the organized thrombi.

In postcapillary pulmonary hypertension, the alveolar septa appeared thickened and congested. This thickening was due to the dilatation of alveolar capillaries, but also, although to a lesser degree, to an apparent increase in the cellular component of alveolar tissue. With trichrome stain, no manifest increase in collagen or elastic fibers was noted. In the alveoli, there was a variable number of hemosiderin-laden macrophages attesting the chronicity of the congestion. The media of the small and medium sized pulmonary arteries was thickened; their intima was occasionally fibrotic. In the cases of veno-occlusive disease, the lumen of the veins was occluded by a loose connective tissue containing elongated cells that were disposed around the narrowed vascular lumen. These cells were stained with alpha-SM actin antibody (Figure 3a and b).

Immunohistochemical studies showed that many cells in alveolar septa stained with alpha-SM actin antibodies (Figures 1k through m and 4a through c). These cells were particularly abundant in cases of mitral stenosis as well

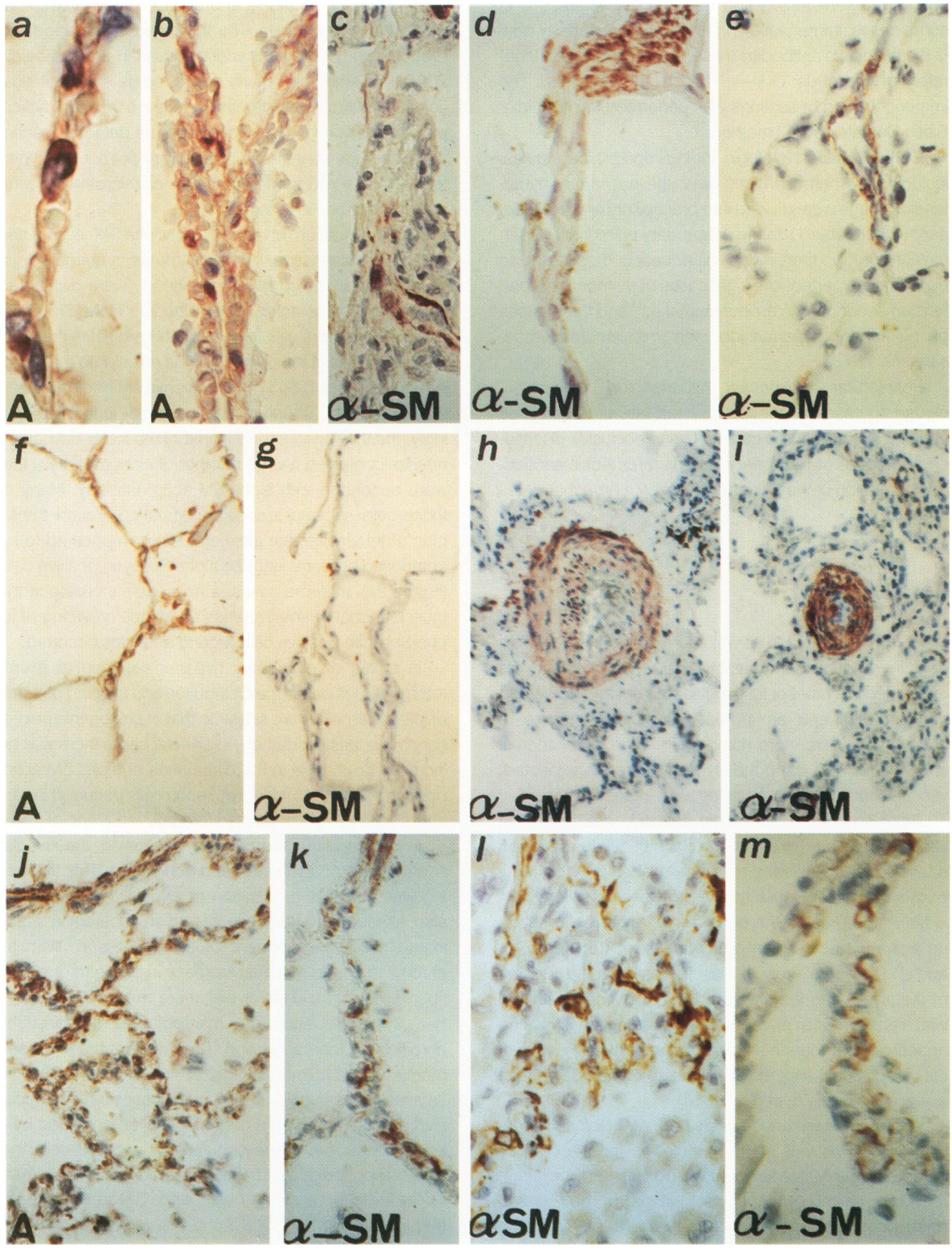
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Figure 1. *Immunohistochemical identification of alveolar myofibroblasts (contractile interstitial cells) and of alpha-SM actin-positive myoid cells. A = anti-actin antibody, alpha-SM = anti-alpha-SM actin antibody stainings.* **a–e**: *Normal lung;* **f–i**: *precapillary PTH;* **j–m**: *postcapillary PHT.* **a**: *Myofibroblasts stained by anti-actin in rat alveolar septum (1000×).* **b**: *Myofibroblasts stained by anti-actin in human alveolar septum (500×).* **c**: *Alpha-SM actin-labeled cells around a postcapillary venule (pericytes) in human lung. Note the absence of alpha-SM actin labeling in alveolar septal cells (400×).* **d**: *Muscle cells located at the opening of an alveolus into an alveolar duct; human lung (600×).* **e**: *A postcapillary venule encircled by a rim of alpha-SM actin-labeled cells (pericytes); human lung (500×).* **f**: *Alveolar tissue in precapillary PHT (pulm.art.press. 92/55 mm Hg - see also Figure 2, same case). Note labeling of septal cells by anti-actin antibody (200×).* **g**: *Same case as* **f**. *Note the absence of alpha-SM actin-labeled cells in alveolar tissue (200×).* **h**: *+ i) Aminorex-induced pulmonary hypertension (pulm.art.press. 120/75 mm Hg). Thickened muscular coat and intimal cells of different-sized arteries are stained with alpha-SM actin. No alpha-SM actin-positive cells are visible in the septa (250×).* **j**: *Veno-occlusive disease (pulm.art.press. 73/50 mm Hg, same case as Figure 3). Numerous actin-labeled interstitial cells are present in alveolar septa (250×).* **k**: *Same case as letter j. Presence of numerous alpha-SM actin-laden cells in alveolar septa (250×).* **l**: *Mitral stenosis. Alpha-SM actin antibody-laden cells in the alveolar septa. Note the linear structures partially underlining the capillary walls and presumably corresponding to the cytoplasmic processes of cells (500×).* **m**: *Congestive heart failure. Note the linear wire stringlike labeling of cells (600×).*

as in veno-occlusive disease (Figure 1j and k). On high magnification, they appeared to be in close proximity of one or the other wall of the capillaries, and thus exhibited a sinusoidal disposition in the alveolar septa (Figures 1k through m and 4a through c). Often, long cytoplasmic extensions of interstitial cells appeared as a thin cordlike structure underlining the walls of the capillary. In lungs with chronic passive congestion due to left ventricular failure, immunostaining properties of alveolar interstitial cells were similar to those in mitral stenosis and in veno-occlusive disease; namely, the cells were decorated with general actin as well as with alpha-SM actin antibodies. With the latter antibody, they displayed the same sinusoidal disposition between the capillary network (Figure 1l and m) as do the alveolar myofibroblasts, ie, CIC.[1–3] However, we had the impression that, in some cases of left ventricular failure, although there were always many cells labeled with alpha-SM actin antibodies, they were fewer than in cases of mitral stenosis or in veno-occlusive disease.

Discussion

Our results demonstrate that alveolar septa of normal human lungs are devoid of alpha-SM actin-laden cells; such cells are present only around venules and in muscle rings at the opening of air sacculi into the alveolar ducts. This distribution is similar to what has been reported recently in normal rat lungs.[25] The alpha-SM actin-decorated cells around the venules correspond very probably to pericytes. In our experience, detection of alpha-SM actin in alveolar tissue allows discrimination between myofibroblasts and pericytes (Kapanci and Ribaux, unpublished data). Using electron microscopic immunochemistry, it has in fact been demonstrated recently that this isoform of actin occurs in pericytes, where it is localized in the filamentous bundles.[31] Hence, absence of alpha-SM actin-laden cells in normal alveolar septa raises the question whether pericytes do really exist at the level of alveolar capillaries, as it has been claimed previously.[32,33] According to our findings, morphologically typical pericytes occur at alveolar tissue only around postcapillary venules. The alpha-SM actin-containing cells at the opening of air sacculi into the alveolar ducts correspond to the sphincterlike structures—alveolar entrance rings; these structures are thought to regulate the air flow into the alveoli.[34]

The purpose of our study was to investigate a situation in which alveolar tissue changes was known to be related to hemodynamic factors. We found that, in precapillary hypertension, no matter its etiology, idiopathic, secondary to aminorex intake,[29] or to repeated microthromboembolisms,[30] the contractile element content in interstitial cells does not change, and furthermore no alpha-SM actin-laden cells occur in the alveolar tissue. The small arteries show, however, some interesting modifications: the muscle coat seems to extend into the walls of smaller and more peripheral vessels as reported previously in pulmonary hypertension[35]; moreover, in the thickened intima as well as in plexiform lesions, many cells are decorated by alpha-SM actin antibodies, thus confirming their smooth muscle cell nature.[35–38]

In postcapillary pulmonary hypertension, that is, in chronic pulmonary congestion, many cells in the alveolar interstitium appear to be decorated by alpha-SM actin antibody. These cells have the same situation and disposition as the common alveolar myofibroblasts.[1] Such a similarity raises the question whether alveolar myofibroblasts in normal alveolar septa and alpha-SM actin-positive cells in pulmonary postcapillary hypertension are related cells.

It is worth reminding that originally the concept of myofibroblast (including pulmonary CIC) had been developed on the basis of ultrastructural criteria.[1,3–6,16] Such cells have been identified in several normal organs,[1,6,16,39,40] and their *ex novo* appearance has been shown in many pathologic conditions such as granulation tissue, connective tissue, or parenchymal organ retractions and fibromatoses.[4,5,7,39] Although these cells have been the object of many studies, their exact nature and origin remain obscure.

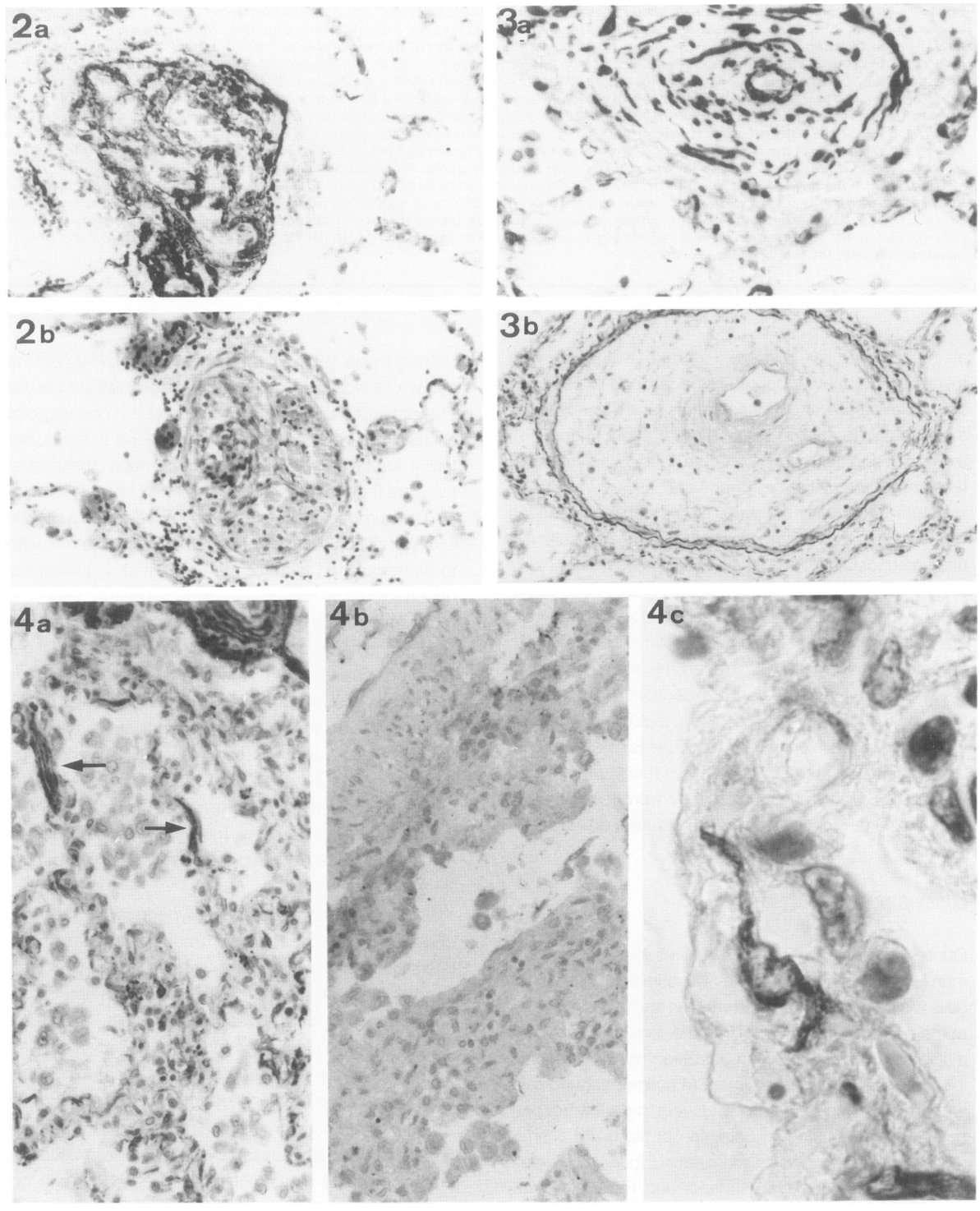

Figure 2. *Plexiform lesion.* **a:** *Cells forming the obliterative canalized intravascular nodules are deeply stained by alpha-SM actin antibody (alpha-SM actin, 200×).* **b:** *Same lesion stained with H & E few sections further (HE, 200×).* **Figure 3.** *Veno-occlusive disease.* **a:** *Many alpha-SM actin-laden cells are present in the obliterative intimal proliferation of the vein (alpha-SM actin, 250×).* **b:** *Same vessel stained with elastica stain. Note the peculiar aspect of obliterative lesions in the veno-occlusive disease (VGE, 200×).* **Figure 4.** *Alveolar interstitium in chronic passive congestion:* **a:** *Note the labeling of many interstitial cells with alpha-SM actin antibody appearing as black dots or lines (staining of the arterial wall serves as a positive control). Arrows point to alveolar duct muscle fibers (alpha-SM actin, 250×).* **b:** *A control section from the same lung. No labeling of the interstitium nor of the arterial wall is visible (mouse IgG, 250×).* **c:** *An interstitial cell (myofibroblast) in an alveolar septum stained with alpha-SM actin antibody; it possesses cordlike cytoplasmic projections insinuating between capillaries (alpha-SM actin, 1600×).*

Thanks to biochemical[41] and recent immunohistochemical[17,18] approaches, it has been shown that, apart from a few exceptions,[16,17,20] stroma cells of normal organs do not contain smooth muscle cell markers,[16–19] while such markers appear in cells rather commonly in pathologic situations.[18,19] When in fibroblastic cells, we detect presence of actin filaments, we refer to them with a generic name: 'myofibroblasts.'[4,5] However, it is now shown that, depending on the topography of the cell and the nature of the disease, such myofibroblasts may develop different cytoskeletal features despite the fact that they all have the same ultrastructural appearance. Thus, myofibroblasts were shown to contain vimentin and cytoplasmic actins (V cells), vimentin and alpha-SM actin (VA cells) or vimentin, alpha-SM actin, and desmin (VAD cells).[19] Such variations are present in hypertrophic scars, in different fibromatoses, and in stromal reactions in tumors[18,19,23]; they suggest that fibroblasts and smooth cells may modulate toward an intermediate type of cell or possibly one into the other.[19]

In alveolar tissue, proliferation of alpha-SM actin-laden cells has been previously reported in bleomycin-injured rat lungs.[25] These cells correspond to the VA type of myofibroblasts.[19] They occur in fibrotic areas as well as in alveolar septa at the edge of such lesions. Although the origin of these cells is not clear, Mitchell et al[25] favor the possibility of alveolar interstitial cells or perhaps the pericytes, as being their progenitors.

The immunostaining properties of alveolar myofibroblasts that we observe in postcapillary pulmonary hypertension is similar to those reported by Mitchell et al[25] in bleomycin-induced lung fibrosis; they correspond to VA type of myofibroblasts.[19] The location of these cells and their topography in alveolar septa are similar to those of V-type normal alveolar myofibroblasts. Hence it is tempting to propose V-type alveolar myofibroblast as a progenitor cell to VA-type myofibroblasts. Whether the mechanical stretch of alveolar capillaries is responsible for such a modulation of alveolar myofibroblasts remains yet to be proven. In the skin, the effect of stretch on the organization of microfilamentous and intermediate filaments has been shown previously.[42] Recently it has also been shown that, during pulmonary hypertension in the newborn, intimal cells in small pulmonary arteries express alpha-SM actin.[43] It is conceivable that, in the lung parenchyma, mechanical stimuli also induce the modulation of V-type myofibroblasts into the VA type. However, to confirm this hypothesis, some experimental work on animal models of pulmonary hypertension is necessary.

If, in lungs, mechanical stress in certain situations may be a stimulus for myofibroblast modulation, this is certainly not the only mechanism involved in muscle cell proliferation. Indeed, in idiopathic pulmonary fibrosis leading to honeycombing, proliferation of well-established muscle cells is known to occur.[44,45] In some cases, this muscular hyperplasia may reach such an extent that, in older literature, it has been referred to as 'muscular cirrhosis' of the lung.[46,47] The disposition of 'muscle' in these advanced conditions is different from what we have observed in postcapillary pulmonary hypertension. In muscular cirrhosis of the lung, the muscle is oriented in a totally disorganized fashion. Fascicles of muscle cells are layed down in scarlike fibrotic areas, as well as in peribronchosaccular connective tissue or along the alveolar ducts.[47]

These features suggest that, in such conditions, the muscle is derived at least partly from alveolar ducts[47] and vessels, representing a phenomenon of hypertrophy and/or hyperplasia of pre-existing smooth muscle. In bleomycin-induced lung fibrosis in the rat, participation of pre-existing muscle tissue in the muscularization of the lung process has been suggested by Mitchell et al.[25]

In conclusion, it should be emphasized that at present we do not know the pathogenesis of the muscularisation of lung tissue. There are certainly different initiating factors, but the mechanisms that lead to increased amounts of cells with smooth muscle features during these situations are not clear. Possibly, mechanical factors induce modulation of myofibroblasts in some situations. In other situations, such as idiopathic pulmonary fibrosis,[48] macrophage-associated factors may stimulate the proliferation of pre-existing muscle cells. Further studies will be necessary to elucidate the role of different intracellular and extracellular factors in the development of muscular differentiation of lung cells.

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