Histopathology 2004, **44**, 156–163

Clear-cell proliferation of the lung with lymphangioleiomyomatosis-like change

S A Pileri, A Cavazza,[1] M Schiavina,[2] M Zompatori,[3] M Pederzoli,[2] M Goldfischer,[5] E Sabattini, S Ascani, G Pasquinelli,[4] F Bonetti[6] & T V Colby[7]

Chair of Pathologic Anatomy, Institute of Haematology and Clinical Oncology 'L. e A. Seràgnoli', Bologna University, Bologna, [1]Unit of Pathologic Anatomy, St Maria Nuova Hospital, Reggio Emilia, [2]Unit of Lung Physiopathology, St Orsola Hospital, Bologna, [3]Chair of Radiology, Parma University, Parma, and [4]Division of Ultra-structural Pathology, St Orsola Hospital, Bologna, Italy, [5]Department of Pathology, Hackensack University Medical Center, Hackensack, NJ, USA, [6]Institute of Pathologic Anatomy, Verona University, Verona, Italy, and [7]Department of Pathology, Mayo Clinic Scottsdale, Scottsdale, AZ, USA

Date of submission 27 May 2003
Accepted for publication 27 June 2003

Pileri S A, Cavazza A, Schiavina M, Zompatori M, Pederzoli M, Goldfischer M, Sabattini E, Ascani S, Pasquinelli G, Bonetti F & Colby T V

(2004) *Histopathology* **44**, 156–163

Clear-cell proliferation of the lung with lymphangioleiomyomatosis-like change

Aims: To describe two cases of a peculiar pulmonary lesion, which expand both the morphological and the immunophenotypic spectrum of perivascular epithelioid cell (PEC)-related disorders.

Methods and results: One man and one female, with and without the tuberous sclerosis complex (TSC), respectively, showed pulmonary cysts and small nodules on computed tomography scan. In the former, lymphangioleiomyomatosis (LAM) was suspected. In both cases, an open lung biopsy was performed, whose cut surface displayed numerous cysts lined by thin/thick septa. Microscopically, the septa were associated with micronodular or interstitial proliferation of medium/large-sized elements with abundant clear (periodic acid–Schiff-positive/diastase-sensitive) cytoplasm and distinct cell borders, embedded in fibrous tissue. The elements were CD34+, vimentin-positive and, to a lesser extent, HMB-45+ and MART-1+. The stains for specific muscle actin, desmin, S100 protein, CD31, FVIIIRAg, cytokeratins, CD45, CD68, oestrogen and progesterone receptors were all negative. Ki67 labelling was <1%. Electron microscopy displayed cytoplasmic vacuoles containing glycogen particles. The *TSC1* and *TSC2* gene status could not be assessed because of poor DNA preservation. In the man with TSC, a focus of micronodular pneumocyte hyperplasia was also found.

Conclusions: Because of the coexpression of CD34 and melanoma-associated antigens and the occurrence of TSC in one patient, the cases described here add a new piece to the puzzle of PEC lesions and contribute to the open discussion on the origin of LAM and LAM-like proliferations.

Keywords: lymphangioleiomyomatosis, clear-cell 'sugar' tumour of the lung, perivascular epithelioid cell, tuberous sclerosis complex, morphology, phenotype, molecular biology

Abbreviations: AML, angiomyolipoma; LAM, lymphangioleiomyomatosis; PEC, perivascular epithelioid cell; TSC, tuberous sclerosis complex

Introduction

Pulmonary lymphangioleiomyomatosis (LAM) is an uncommon disease characterized by multifocal proliferation of peculiar smooth muscle cells, with secondary cystic changes, occurring sporadically or in association with the tuberous sclerosis complex (TSC).[1–9] It is part of a family of lesions, many TSC-related, characterized

Address for correspondence: Dr Alberto Cavazza, Unità Operativa di Anatomia Patologica, Arcispedale S. Maria Nuova, Viale Risorgimento 80, 42100 Reggio Emilia, Italy. e-mail: cavazza.alberto@asmm.re.it

by the proliferation of distinctive cells [perivascular epithelioid cells (PEC)], which show coexpression of muscle and melanocytic markers and a propensity for a perivascular distribution.[2,3,10–16] Besides LAM, this family includes: angiomyolipoma (AML) and its morphological variants (leiomyoma-like AML, lipoma-like AML, oncocytoma-like AML, monotypic epithelioid AML), pulmonary and extrapulmonary clear-cell 'sugar' tumours, lymphangiomyoma, and renal capsuloma.[3,15] PECs can undergo morphological modulation, their shape varying from epithelioid to spindly and their cytoplasm from clear to granular eosinophilic.[3,16] Such a modulation is mirrored by phenotypic variability: thus, lesions composed of spindle cells only display strong immunoreactivity for actin and focal positivity for melanoma-associated antigens, while a pure epithelioid growth reveals the opposite phenotypic profile.[3] Importantly, as Bonetti *et al*. have pointed out: 'not all morpho-phenotypical stages of PEC are well defined; in particular, the ends of the spectrum are in need of additional study'.[3]

In line with these concepts, the spectrum of pulmonary lesions associated with LAM and TSC has been broadening, now comprising micronodular pneumocyte hyperplasia, AML, and clear-cell tumours.[2–5,7,9,11,17,18] In addition, the differential diagnosis of LAM has recently been expanded by Hironaka and Fukayama, who described a 27-year-old woman without stigmata of TSC, presenting with a clear-cell tumour of the lung and a peculiar interstitial proliferation of HMB-45+ clear cells with a secondary cystic (LAM-like) change.[19]

Here, we report two patients who presented radiologically with multiple pulmonary cysts and nodules. At the microscopic level, there was a micronodular/interstitial proliferation of clear cells, in the absence of clear-cell tumour. One of these cases, a man with TSC, simulated the clinico-radiological pattern of LAM and there was associated micronodular pneumocyte hyperplasia. Most interestingly, besides the expected positivity for melanoma-associated antigens in the interstitial clear cells, both cases were characterized by the expression of CD34, in the absence of muscle-specific actin. These findings, undetected in previous studies, expand the spectrum of our knowledge of LAM and LAM-like lesions, as well as of the phenotypic profile of PEC-related disorders.

Patients and methods

CASE REPORTS

Case 1

A 26-year-old non-smoking man was affected by TSC, which had manifested with recurrent seizures, schizophrenia, mental retardation, endoventricular astrocytoma, multiple bilateral renal AMLs, cystic hamartoma of the 8th hepatic segment, frontal sebaceous adenoma, lumbar shagreen skin patch, and round peri-ungual fibromas. He was a phenotypically normal male with regularly developed external genitalia, sexual hair and body fat distribution, and no gynaecomastia. The patient had no pulmonary symptoms or signs. Arterial blood gas analysis on room air and spirometry tests showed normal values. A standard chest X-ray was normal. A thoracic high-resolution computed tomography (CT) scan (Figure 1) showed a few air-filled pulmonary cystic lesions, up to 10 mm

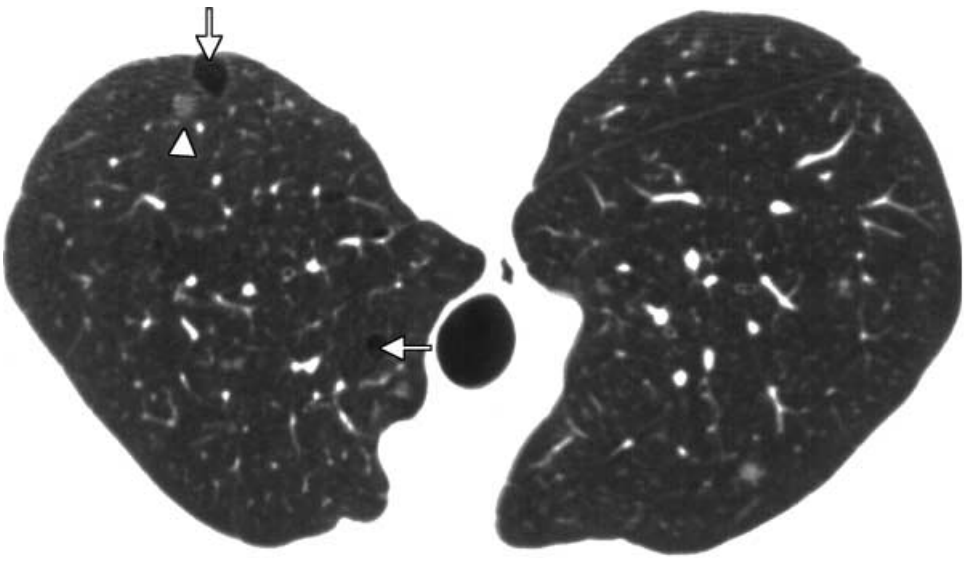

Figure 1. Case 1: High-resolution computed tomography scan at the upper lobe level. Presence of a few cystic lesions with thin walls and uniform size (arrows), along with small hazy nodules (arrowhead).

in diameter, with thin and regular walls. The cysts showed no preferential distribution and were associated with approximately 10 small round nodules, randomly distributed at the periphery of both lungs. The nodules were not located in the centrilobular areas, presented soft tissue density, and were sharply outlined, with diameters ranging from 5 to 10 mm. Because of the clinical suspicion of LAM, multiple video-assisted thoracoscopic biopsies of the left lower and upper lobes were performed. No treatment was instituted. Twenty months after the diagnosis, the patient is still alive and well; no significant changes have been recorded at repeated thoracic high-resolution CT scans.

Case 2

A 61-year-old asymptomatic female smoker with no signs of TSC. A standard chest X-ray, performed during the work-up for repair of carpal tunnel syndrome, revealed a small nodule on the lower lobe of the left lung. Laboratory findings and spirometry tests were normal. A thoracic CT scan showed cystic changes in both lung fields, particularly on the left side. At the edge of a bleb, in the upper lobe of the left lung, there was a nodule, 9 mm across, neither cavitated nor calcified. The heart, mediastinum and pleura were normal. A left thoracotomy with resection of a bleb and the nodule was performed. The postoperative period was uneventful and the patient is alive and well 18 months after surgery.

METHODS

Case 1 was sent fresh in isotonic solution, while case 2 was immediately placed into fixative and processed routinely. The former was divided into two parts: one half was cryopreserved in liquid nitrogen and then stored at −80°C, while the other underwent routine procedures. The latter consisted of fixation in 10% buffered formalin for 24 h, dehydration in graded alcohols, clearing in Histoclear, and embedding in Merck paraffin at 56°C. Sections (3 μm thick) were cut from paraffin blocks of both cases and stained according to the following methods: haematoxylin and eosin (H–E), periodic acid–Schiff (PAS), PAS with diastase predigestion, Prussian blue for iron, Mallory's trichrome, and Gomori silver impregnation for reticulin fibres. Additional sections were cut and coated on electrically charged slides and stored at 40°C for 2 h. Following rehydration, they underwent antigen retrieval in 1 mM EDTA (pH 8.0) or citrate buffer (pH 6.2) in a microwave oven (two to four cycles of irradiation at 750–900 W, lasting 5 min each), according to previously reported methods.[20] Subsequently, the sections were incubated with the following antibodies for 30 min at room temperature: anti-cytokeratins (clones MNF116, 35βH11, and 34βE12), anti-desmin (clone D33), anti-muscle actin (clone HHF35), anti-vimentin (clone V9), anti-protein S100 (polyclonal), anti-melanoma associated antigens (clones HMB-45 and MART-1), anti-CD45 (clones 2B11 + PD7/26), anti-CD34 (clone QBEND10), anti-CD31 (clone JC/70 A), anti-FVIIIRAg (polyclonal), anti-CD68 (clone PG-M1, kindly provided by Professor B. Falini, Universitá di Perugia, Perugia, Italy), anti-oestrogen receptor (clone 1D5), anti-progesterone receptor (clone 1A6), and anti-nuclear proliferation-associated antigen Ki67 (clone MIB-1). The antibodies—all purchased from Dako (Glostrup, Denmark)—were detected by the alkaline phosphatase–antialkaline phosphatase (APAAP) technique or the Labelled streptavidin blotin (LSAB) method.[21] Most of the immunohistochemical tests were automatically performed on a TechMate 500 immunostainer.

For electron microscopy, tissue fragments of tumours in both cases were retrieved from paraffin blocks, de-waxed, rehydrated, fixed in 1% buffered osmium tetroxide and embedded in epoxy resin. Hemi-thin sections showed representative tumour architecture in case 2 only; consequently case 1 was lost for ultrastructural investigation. Thin sections from case 2 were stained with uranyl acetate and lead citrate, and observed in a Philips 410 transmission electron microscope.

DNA was extracted from both the cryopreserved sample of case 1 (the routine material having been exhausted) and the paraffin block of case 2 in order to perform loss of heterozygosity analysis of the *TSC1* and *TSC2* genes as previously reported.[22]

Results

PATHOLOGICAL FINDINGS

At macroscopic examination, the open lung biopsies obtained from both patients were characterized by the presence of cystic changes with thin or thick septa apparent on the cut surface. On light microscopy, the septal structures of the cysts consisted of micronodular and diffuse proliferations of medium to large-sized cells, with abundant clear cytoplasm and distinct cytoplasmic borders embedded in fibrous tissue (Figure 2). The nuclei were round to oval with inconspicuous nucleoli (Figures 2b and d). The cytoplasm of the cells was intensively positive with PAS, which was sensitive to diastase predigestion. In some areas, which were more numerous in case 2, clear cells were located within an abundant eosinophilic hyaline fibrous matrix (Figure 2b). The septal structures were partially

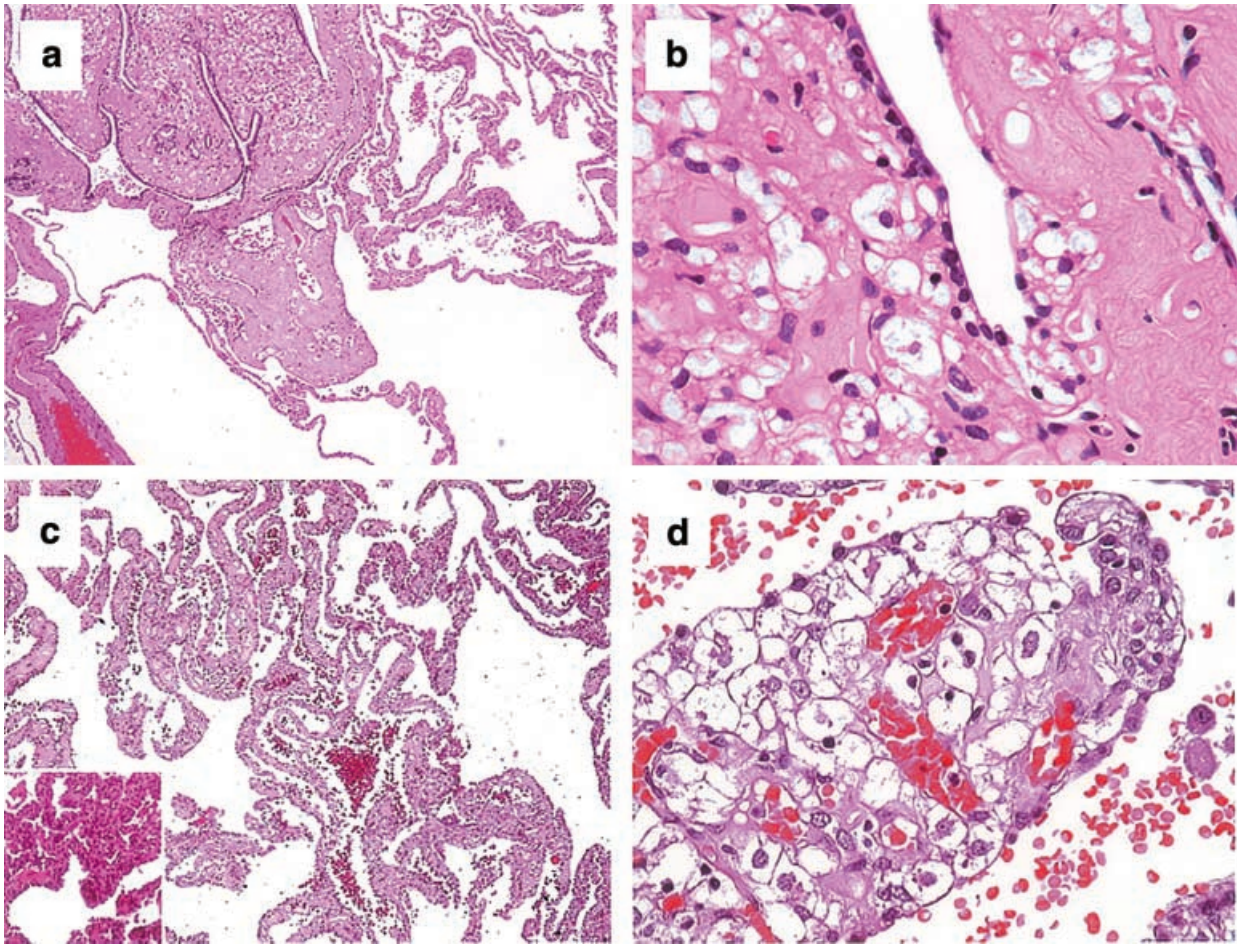

Figure 2. **a,** Case 2: Interstitial micronodules and cystic change observed at low power. **b,** Case 2: The nodules consist of medium–large sized cells with a large rim of clear cytoplasm; these cells are at times located within hyaline fibrous stroma. **c,** Case 1: At low power, thick septal structures and abnormal, variably sized cystic air spaces can be seen. Inset: a focus of micronodular pneumocyte hyperplasia is shown. **d,** Case 1: Details of the lesion at higher magnification.

covered by cuboidal alveolar cells (Figure 2d). Occasional iron-loaded macrophages and a focus of micronodular pneumocyte hyperplasia were detected in case 1 (Figure 2c). No proliferation of spindle-shaped smooth muscle cells, mitotic figures or necrosis was seen.

IMMUNOHISTOCHEMICAL, ELECTRON MICROSCOPIC AND MOLECULAR FINDINGS

On phenotypic analysis, the clear cells were strongly positive for vimentin and CD34 (Figures 3a and b) and showed partial expression of the melanoma-associated antigens HMB-45 and MART-1 (Figure 3c). They were negative for muscle-specific actin, desmin, S100 protein, CD31, FVIIIRAg, cytokeratins, CD45, CD68, and oestrogen and progesterone receptors (Figures 3b, d and e). Ki67 labelling was <1%.

On electron microscopy, the clear cells showed large cytoplasmic collections of rimmed vacuoles containing glycogen particles (Figure 3f); organelles, mainly mitochondria and strands of rough endoplasmic reticulum, were scarce; clusters of round, elongated membrane-bound dense granules (Figure 3g) were focally found.

Molecular studies unfortunately did not provide useful information: in fact, the frozen block did not contain the lesion, while the DNA extracted from case 1 was partly degraded, thus preventing evaluation of the *TSC1* and *TSC2* genes.

Discussion

The two cases described here show several points of interest. On pathological grounds, they pose the

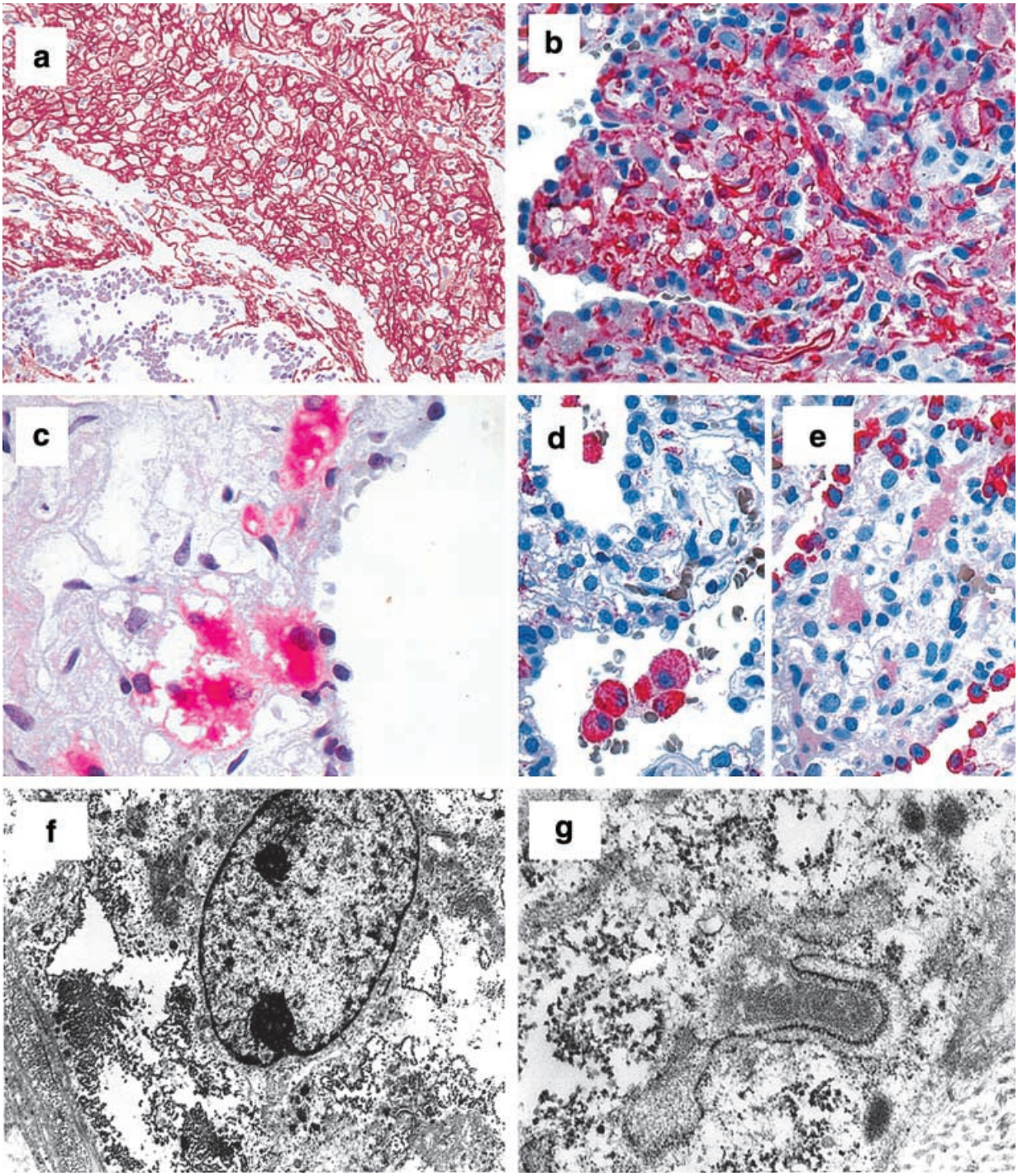

Figure 3. a, Case 2: Strong expression of CD34 by clear cells. LSAB-peroxidase method; monoclonal antibody QBEN10; Mayer's haematoxylin nuclear counterstaining. **b,** Case 1: Clear cells express CD34. APAAP technique; QBEN10 monoclonal antibody; Gill's haematoxylin counterstaining. **c,** Case 2: Most cells express the MART-1 molecule. APAAP technique; Melan A monoclonal antibody; Gill's haematoxylin nuclear counterstaining. **d,** Case 1: The anti-CD68 monoclonal antibody stains only some macrophages contained within the residual alveolar cavities, but does not react with clear cells. APAAP technique; PG-M1 monoclonal antibody; Gill's haematoxylin nuclear counterstaining. **e,** Case 1: One of the nodules is lined by scattered cytokeratin-positive pneumocytes; clear cells are unstained. APAAP technique; MNF116 monoclonal antibody; Gill's haematoxylin nuclear counterstaining. **f,** Case 2: Portion from a septal clear cell showing rimmed vacuoles with glycogen particles and clusters of dense bodies in the cytoplasm. **g,** Ultrastructural detail of the tumour cell cytoplasm showing membrane-bound dense bodies probably representing premelanosomes.

problem of their relationship with LAM, contribute to the existing debate on pulmonary clear-cell proliferation with cyst formation, and document CD34 as a novel immunophenotypic expression of PEC-related disorders. On clinical grounds, they suggest the existence of a further pulmonary change associated with TSC, possibly with a very indolent course. In addition, they illustrate that within the spectrum of TSC-related lung lesions not all cysts and micronodules correspond to typical LAM and micronodular pneumocyte hyperplasia, respectively.

LAM is a rare entity, which has been the object of numerous reports in the literature (for comprehensive reviews of the topic see Refs 1–3,6,9). It almost exclusively affects females of childbearing age, only one convincing case having been recorded in a male patient.[23] Chest radiology shows a reticulonodular pattern or a honeycomb lung in advanced cases.[2,6,9] The diagnosis is made on open lung or, more rarely, transbronchial biopsies, although high-resolution CT may be sufficient in typical cases.[2,6,9,10] Microscopically, LAM is characterized by interstitial, peribronchial, perivascular, and perilymphatic micronodular proliferation of smooth muscle cells with epithelioid, clear or fusiform appearance, producing cystic lesions and frequent lung haemosiderosis.[2] Phenotypically, LAM cells express vimentin, muscle-specific actin, desmin, and the melanoma-associated antigens HMB-45 and MART-1.[1–4,10–12,14] Ultrastructural studies reveal microfilament bundles with electron-dense condensations, glycogen granules, and membrane-bound dense bodies interpreted as premelanosomes.[2]

In the last few years, special interest has been aroused by the relationships between LAM and TSC.[1–5,7–9] In fact, LAM can present either as an isolated disorder (sporadic LAM) or in association with TSC. Thus, LAM might represent a *forme fruste* of TSC, as also suggested by the recent detection of loss of heterozygosity and/or mutations of the TSC gene *TSC2* in patients with sporadic LAM.[3–5,8,9] In line with this hypothesis, LAM has been found in association with micronodular pneumocyte hyperplasia, a lesion frequently observed in TSC patients, and, more rarely, with clear-cell tumours.[2,3,5,7,17,18,24] Interestingly, LAM, AML and clear-cell tumour express melanoma-associated antigens, a finding suggesting a common PEC derivation.[2,3,10–12,14]

Recently, this spectrum of TSC-related pulmonary lesions has been further expanded. In 1995, Chiodera *et al.* reported a young female with TSC, who showed no pulmonary disease (clinically, radiologically or macroscopically) and died of unrelated causes.[24] Microscopically, at autopsy, the lungs revealed a diffuse 'miliariform' proliferation of epithelioid clear cells with the phenotype of PEC. This unique lesion was tentatively interpreted as an early manifestation of LAM. In 1999, Hironaka and Fukayama described a 27-year-old woman without TSC, presenting with a clear-cell tumour associated with a peculiar interstitial proliferation of HMB-45+ clear cells in a LAM-like distribution.[19] The authors argued that the observed pattern might represent 'a particular pulmonary lesion consisting of clear-cell tumour–LAM hybrid cells', although the possibility that the interstitial component reflected 'a peculiar mode of spreading of clear-cell tumour', could not be ruled out. Chuah and Tan had previously reported a similar micronodular interstitial proliferation of clear cells, associated with LAM and micronodular pneumocyte hyperplasia, in a 33-year-old female with TSC.[17]

The present two cases are speculatively challenging and possibly add new pieces of information to the puzzling subject of TSC-related pulmonary lesions. They both were characterized by an interstitial proliferation of clear elements, which produced pulmonary cysts and micronodules, but, in contrast to the cases of Chuah and Tan and Hironaka and Fukayama,[17,19] lacked the simultaneous occurrence of typical LAM and clear-cell tumour, respectively. One of our patients had overt TSC. The abnormal interstitial cells displayed the classical morphological, electron microscopic and immunohistochemical features of PEC.[2,3,11] However, besides the expected positivity for melanoma-associated antigens (i.e. HMB-45 and MART-1), they stained strongly for CD34. The latter molecule, which is regularly expressed by haematopoietic stem cells, endothelia and stromal cells as well as by tumours derived from them,[25–29] has not been previously detected in PEC-related elements. CD34 staining was in fact limited to vascular structures in four large series of PEComas, recently reported in the literature.[15,30–32] Thus, either CD34 should be added to the list of possible antigens expressed by PEC or the presently reported cases derive from a primitive pluripotential mesenchymal cell ancestral to the PEC compartment. Prospective and retrospective studies on large series of cases are mandatory to clarify the biological significance and the real incidence of CD34 positivity among PEC-related lesions.

Another interesting finding of the present study refers to the clinico-radiological presentation in patient 1, a male affected by TSC, in whom the pulmonary cysts closely simulated the high-resolution CT pattern of LAM. This observation suggests that, besides classical LAM, interstitial clear-cell proliferation can represent another rare cause of pulmonary cysts and

micronodules in individuals with TSC. In the light of this, the detection of cysts and nodules should be interpreted with some caution in prospective high-resolution CT screening studies in TSC patients, since cysts and micronodules might not necessarily correspond to LAM and micronodular pneumocyte hyperplasia, respectively, as suggested by Franz *et al.*[5] Prospective pathological studies are desirable in order to clarify this issue.

The prognosis of the lesion here described appears favourable, at least based on the limited follow-up period of the two cases. Clearly, larger series of patients should be gathered in order to draw any conclusion as to whether the condition differs prognostically from classical LAM.

On practical grounds, the present condition should be differentiated from several pathological disorders other than LAM, which may occasionally grow in the lung interstitium and be associated with secondary cystic changes. These include metastatic involvement by low-grade sarcomas (e.g. endometrial stromal sarcoma and dermatofibrosarcoma protuberans) or the recently described PEComa of the uterus.[16] Due to CD34-positivity, epithelioid haemangioendothelioma should also be regarded as a potential diagnostic pitfall.[29] A basic contribution to the diagnosis is, however, provided by the coexpression of melanoma-associated markers and CD34 in the absence of positivity for muscle-specific actin, desmin, S100 protein, CD31, FVIIIRAg, cytokeratins, CD45, CD68, and oestrogen and progesterone receptors. This phenotypic profile differs completely from that of each of the above-mentioned tumours.[16,26,29,33]

In conclusion, the present report deals with two patients with pulmonary cysts and micronodules, in one case simulating the high-resolution CT pattern of LAM. The lesions were caused by a peculiar interstitial proliferation of clear cells, which coexpressed melanoma-associated antigens and CD34, an unprecedented phenotypic finding. In general, the two cases might be interpreted as LAM with unusual morphological findings and phenotypic profile. However, some features do not fit with the classical description of the disease, such as the sex of one of the patients, the age of the female subject, and the distinctive sclerosis observed in both instances. Accrual of further cases is warranted to assess whether the lesion described might represent the missing link between the early manifestation (without clinical signs) of LAM, purely composed of PEC-derived clear cells, originally described by Chiodera *et al.*,[24] and full-blown LAM composed of a mixture of spindle and epithelioid cells disrupting the pulmonary architecture.

References

1. Anonymous. Report of Workshop on lymphangioleiomyomatosis. *Am. J. Respir. Crit. Care Med.* 1999; **159;** 679–683.
2. Bonetti F, Chiodera PL. The lung in tuberous sclerosis. In Corrin B ed. *Pathology of lung tumors.* London: Churchill Livingstone, 1997; 225–239.
3. Bonetti F, Pea M, Martignoni G *et al.* The perivascular epithelioid cell and related lesions. *Adv. Anat. Pathol.* 1997; **4;** 343–358.
4. Carsillo T, Astrinidis A, Henske EP. Mutations in the tuberous sclerosis complex gene *TSC2* are a cause of sporadic pulmonary lymphangioleiomyomatosis. *Proc. Natl Acad. Sci. USA* 2000; **97;** 6085–6090.
5. Franz DN, Brody A, Meyer C *et al.* Mutational and radiographic analysis of pulmonary disease consistent with lymphangioleiomyomatosis and micronodular pneumocyte hyperplasia in women with tuberous sclerosis. *Am. J. Respir. Crit. Care Med.* 2001; **164;** 661–668.
6. Kitaichi M, Nishimura K, Itoh H, Izumi T. Pulmonary lymphangioleiomyomatosis: a report of 46 patients including a clinicopathologic study of prognostic factors. *Am. J. Respir. Crit. Care Med.* 1995; **151;** 527–533.
7. Moss J, Avila NA, Barnes PM *et al.* Prevalence and clinical characteristics of lymphangioleiomyomatosis in patients with tuberous sclerosis complex. *Am. J. Respir. Crit. Care Med.* 2001; **163;** 669–671.
8. Strizheva GD, Carsillo T, Kruger WD, Sullivan EJ, Ryu JH, Henske EP. The spectrum of mutations in TSC1 and TSC2 in women with tuberous sclerosis and lymphangioleiomyomatosis. *Am. J. Respir. Crit. Care Med.* 2001; **163;** 253–258.
9. Urban T, Lazor R, Lacronique J *et al.* Pulmonary lymphangioleiomyomatosis: a study of 69 patients. *Medicine* 1999; **78;** 321–337.
10. Bonetti F, Chiodera PL, Pea M *et al.* Transbronchial biopsy in lymphangioleiomyomatosis of the lung: HMB45 for diagnosis. *Am. J. Surg. Pathol.* 1993; **17;** 1092–1102.
11. Bonetti F, Pea M, Martignoni G *et al.* Clear cell ('sugar') tumor of the lung is a lesion strictly related to angiomyolipoma. The concept of a family of lesions characterized by the presence of the perivascular epithelioid cell (PEC). *Pathology* 1994; **26;** 230–236.
12. Fetsch PA, Fetsch JF, Marincola FM, Travis W, Batts KP, Abati A. Comparison of melanoma antigen recognized by T cells (MART-1) to HMB-45: additional evidence to support a common lineage for angiomyolipoma, lymphangioleiomyomatosis, and clear cell sugar tumor. *Mod. Pathol.* 1998; **11;** 699–703.
13. Jungbluth AA, Iversen K, Coplan K *et al.* Expression of melanocyte-associated markers gp-100 and Melan-A/MAR;T-1 in angiomyolipomas. An immunohistochemical and rt-PCR analysis. *Virchows Arch.* 1999; **434;** 429–435.
14. Matsumoto Y, Horiba K, Usuki J, Chu SC, Ferrans VJ, Moss J. Markers of cell proliferation and expression of melanosomal antigen in lymphangioleiomyomatosis. *Am. J. Respir. Cell. Mol. Biol.* 1999; **21;** 327–336.
15. Tazelaar HD, Batts KP, Srigley JR. Primary extrapulmonary sugar tumors (PEST): a report of four cases. *Mod. Pathol.* 2001; **14;** 615–622.
16. Vang R, Kempson RL. Perivascular epithelioid cell tumor ('PEComa') of the uterus. A subset of HMB-45-positive epithelioid mesenchymal neoplasms with an uncertain relationship to pure muscle tumors. *Am. J. Surg. Pathol.* 2002; **26;** 1–13.
17. Chuah KL, Tan PH. Multifocal micronodular pneumocyte hyperplasia, lymphangioleiomyomatosis and clear cell micronodules of

the lung in a Chinese female patient with tuberous sclerosis. *Pathology* 1998; **30;** 242–246.
18. Flieder DB, Travis WD. Clear cell 'sugar' tumor of the lung: association with lymphangioleiomyomatosis and multifocal micronodular pneumocyte hyperplasia in a patient with tuberous sclerosis. *Am. J. Surg. Pathol.* 1997; **21;** 1242–1247.
19. Hironaka M, Fukayama M. Regional proliferation of HMB-45-positive clear cells of the lung with lymphangioleiomyomatosis-like distribution, replacing the lobes with multiple cysts and a nodule. *Am. J. Surg. Pathol.* 1999; **23;** 1288–1293.
20. Pileri SA, Roncador G, Ceccarelli C *et al.* Antigen retrieval techniques in immunohistochemistry: comparison of different methods. *J. Pathol.* 1997; **183;** 116–123.
21. Sabattini E, Bisgaard K, Ascani S *et al.* Envision Plus: a new immunohistochemical method of choice for diagnostics and research. Critical comparison with the APAAP, ChemMate, CSA, LABC, and SABC techniques. *J. Clin. Pathol.* 1998; **51;** 506–511.
22. Martignoni G, Bonetti F, Pea M, Tardanico R, Brunelli M, Eble JN. Renal disease in adults with TSC2/PKD1 contiguous gene syndrome. *Am. J. Surg. Pathol.* 2002; **26;** 198–205.
23. Aubry M-C, Myers JL, Ryu JH *et al.* Pulmonary lymphangioleiomyomatosis in a man. *Am. J. Respir. Crit. Care Med.* 2000; **162;** 749–752.
24. Chiodera PL, Pea M, Martignoni G, Bonetti F. Pulmonary lymphangioleiomyomatosis associated with miliariform 'sugar' tumor. *Int. J. Surg. Pathol.* 1995; **2** (Suppl.)**;** 483.
25. Barth PJ, Ebrahimsade S, Hellinger A, Moll R, Ramaswamy A. CD34+ fibrocytes in neoplastic and inflammatory pancreatic lesions. *Virchows Arch.* 2002; **440;** 128–133.
26. Bonish BK, Foreman KE, Gutierrez-Steil C, Nickoloff BJ. Phenotype and proliferation of cultured spindle-shaped cells obtained from normal human skin and lesions of dermatofibroma, Kaposi's sarcoma, and dermatofibrosarcoma protuberans: a comparison with fibroblast and epithelial cells of the dermis. *J. Dermatol. Sci.* 1997; **16;** 52–58.
27. Nakayama H, Enzan H, Miyazaki E, Moriki T, Toi M, Zhang Y. CD34-positive stromal cells and alpha-smooth muscle actin-positive stromal cells in the tumor capsule of skin sweat gland neoplasms. *Pathol. Int.* 2002; **52;** 25–30.
28. Pileri SA, Ascani S, Piccioli M *et al.* Acute leukaemia: immunophenotyping in bone-marrow routine sections. *Br. J. Haematol.* 1999; **105;** 394–401.
29. Uchimura K, Nakamuta M, Osoegawa M *et al.* Hepatic epithelioid hemangioendothelioma. *J. Clin. Gastroenterol.* 2001; **32;** 431–434.
30. L'Hostis H, Deminiere C, Ferriere JM, Coindre JM. Renal angiomyolipoma. A clinicopathologic, immunohistochemical, and follow-up study of 46 cases. *Am. J. Surg. Pathol.* 1999; **23;** 1011–1020.
31. Makhlouf HR, Ishak KG, Shekar R, Sesterhenn IA, Young DY, Fanburg-Smith JC. Melanoma markers in angiomyolipoma of the liver and kidney. A comparative study. *Arch. Pathol. Lab. Med.* 2002; **126;** 49–55.
32. Matsui K, Tatsuguchi A, Valencia J *et al.* Extrapulmonary lymphangioleiomyomatosis (LAM): clinicopathologic features in 22 cases. *Hum. Pathol.* 2000; **31;** 1242–1248.
33. Oliva E, Clement PB, Young RH. Endometrial stromal tumors: an update on a group of tumors with a protean phenotype. *Adv. Anat. Pathol.* 2000; **7;** 257–281.