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CASE STUDY

Pathomechanisms of cyst formation in pulmonary light chain deposition disease

M. Colombat*, S. Caudroy[#,¶], E. Lagonotte[¶], H. Mal[+], C. Danel[§], M. Stern[ƒ], M. Fournier[+] and P. Birembaut[#,¶]

ABSTRACT: Cystic lung light chain deposition disease (CL-LCDD) is a recently described rare disorder characterised by numerous cysts and diffuse monoclonal nonamyloid light chain deposits surrounded by macrophagic giant cells. The mechanisms responsible for cyst development remain unknown.

The objectives of the present study were to analyse the major components of the pulmonary extracellular matrix in CL-LCDD and to determine the influence of metalloproteinases (MMPs) by comparison with other cystic lung disorders.

A virtually complete degradation of the elastic network was found in CL-LCDD. To a lesser degree, loss of fibrillar and basement membrane collagens was also observed. Macrophagic giant cells expressed MMP-1, MMP-2, MMP-9, MMP-12 and MMP-14 and *in situ* zymography highlighted a strong gelatinolytic activity. As in CL-LCDD, cystic lesions in Langerhans' cell histiocytosis (LCH) and lymphangioleiomyomatosis (LAM) were characterised by the lack of elastic fibres. Similarly, MMP were expressed in CL-LCDD and LCH but the labelled cells were different. In contrast, few MMPs were detected in LAM.

In conclusion, elastolysis is common to cystic lung light chain deposition disease and other cystic lung disorders, suggesting its implication in cyst formation. Moreover, in cystic lung light chain deposition disease, a role of metalloproteinases in elastolysis is strongly indicated by the metalloproteinase expression and activity pattern.

KEYWORDS: Cyst, elastic fibre, light chain deposition disease, lung, matrix metalloproteinases

Monoclonal immunoglobulin (Ig) is produced by a clonal proliferation of B-lymphocytes. It may be directly pathogenic, particularly *via* tissue deposition: light chain deposits are the most common. They are responsible for light chain amyloidosis or light chain deposition disease (LCDD), a term that, by convention, excludes Ig-derived amyloidosis. Systemic LCDD was described by RANDALL *et al.* [1] in 1976. It is a multivisceral disorder presenting with a constant renal involvement [2–4]. Apart from the kidneys, the heart and liver are the most frequently concerned organs [2–4]. Lung involvement is asymptomatic and usually diagnosed at the time of autopsy. Since 1987, various authors have reported 10 cases of nodular LCDD restricted to the lung [5–10]. They may be single or multiple nodules. The nodules were usually an incidental radiological finding. In 2006, the present authors described, in three patients, a new form of LCDD, named cystic lung LCDD (CL-LCDD) [11]. Since then, no similar cases have been reported. The patients had dyspnoea and progressively developed severe respiratory failure requiring lung transplantation. Histological examination of the explanted lungs showed bilateral diffuse monoclonal κ light chain deposits associated with numerous cysts. Lung cysts defined as well-circumscribed airspaces surrounded by a thin wall do not constitute a specific feature of LCDD as they may occur in various disorders, especially Langerhans' cell histiocytosis (LCH) [12] and lymphangioleiomyomatosis (LAM) [13]. The occasional studies dealing with the pathogenesis of cyst formation mention the crucial role of extracellular matrix (ECM) degradation by matrix metalloproteinases (MMPs) [14–16]. The present study was designed to address the mechanisms of cystic lung destruction. The objectives were to analyse the major components of the pulmonary ECM in CL-LCDD and to determine the influence of MMPs by comparison with other cystic lung disorders.

AFFILIATIONS
*Service d'Anatomie Pathologique, Hôpital Tenon,
[+]Service de Pneumologie, Hôpital Bichat,
[§]Service d'Anatomie Pathologique, Hôpital Européen Georges Pompidou, Paris,
[#]Inserm, UMRS 903, IFR53,
[¶]Laboratoire d'Histologie, UFR Médecine, CHU Maison Blanche, Reims, and
[ƒ]Service de Pneumologie, Hôpital Foch, Suresnes, France.

CORRESPONDENCE
M. Colombat
Service d'Anatomie Pathologique
Hôpital Tenon
4 Rue de la Chine
75020 Paris
France
Fax: 33 156017876
E-mail: colombatm@yahoo.fr

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PATIENTS AND METHODS

Patients

From January 1990 to April 2007, four patients (three females and one males, aged 29–37 yrs) underwent a bilateral lung transplant for end-stage respiratory failure related to CL-LCDD at Beaujon (Clichy), Foch (Suresnes) and Pitié-Salpétrière (Paris) Hospitals (all in France). Three of them have been previously reported [11]. Clinical history before lung transplantation was stereotyped, along with pulmonary histological lesions [11]. Histologically, the main finding was the presence of nonamyloid amorphous eosinophilic deposits of monotypic κ light chains widely infiltrating alveolar walls, small airways and vessels. Congo red did not show apple-green birefringence under polarised light and electron microscopy revealed granular electron-dense deposits, thus excluding amyloidosis. The κ light chain nature of the deposits was determined by immunofluorescence on frozen tissue sections. IgG, IgA, IgM and λ chain were not detected. The deposits were surrounded by numerous CD1a-negative giant cells and were associated with cystic lung destruction characterised by emphysematous-like changes and bronchiolar dilatation (fig 1a).

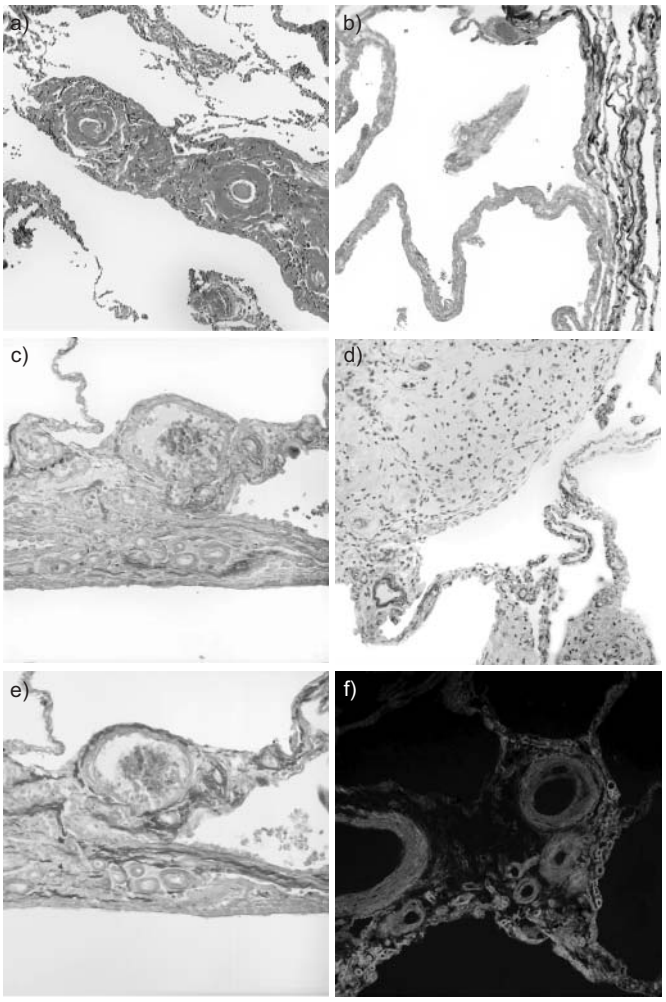

FIGURE 1. Remodelling of extracellular matrix in cystic lung light chain deposition disease and demonstration of gelatinolytic activity. Interstitial amorphous eosinophilic deposits corresponding to monoclonal nonamyloid κ light chains are accumulated in the lung parenchyma. Adjacent to these deposits, emphysematous-like changes are observed (a). Orcein stain shows a complete degradation of elastic fibres in the cystic lung area, contrasting with the preserved lung zone (b). The high-power view focuses on the wall of a cystic airspace developed from a bronchiole. It demonstrates the absence of elastic fibres affecting the vessels and the connective tissue of the lamina propria (c). The amount of basement membranes labelled by type-IV collagen antibody is decreased close to the deposits, compared with that in normal alveolar walls (d). Sirius red highlights the decrease and fragmentation of fibrillar collagens in a dilated bronchiole (e). A strong gelatinolytic activity distributed along vascular basement membranes is detected by *in situ* zymography as green fluorescence (f).

In order to compare CL-LCDD with other cystic lung disorders, three cases of LCH and three cases of LAM were retrieved from the surgical pathology files of Foch Hospital between the years 2006 and 2007.

Tissue samples and methods

Fixed paraffin-embedded tissue were available for each case of CL-LCDD, LCH and LAM. Frozen tissue was only available for three of the four CL-LCDD cases.

ECM analysis used markers for elastic fibres (orcein stain), fibrillar collagens (picrosirius stain) and basement membranes (collagen IV immunostaining; table 1) since elastin and collagen are the major components of lung connective tissue [20].

Immunohistochemistry (table 1) using standard streptavidin-biotin peroxidase methodology (LSAB2 Kit; DAKO, Glostrup, Denmark) after overnight antigen retrieval was performed to study the expression of MMPs (MMP-1, MMP-2, MMP-9, MMP-12 and MMP-14) implicated in the proteolysis of the above-cited ECM components. Immunodectection of tissue inhibitors of metalloproteinases (TIMPs) was also performed (TIMP-1 and -2). Positive and negative controls were run in parallel with appropriate labelling.

In situ zymography was carried out in order to detect and localise gelatinolytic activity of MMP-2 and MMP-9. It was performed on frozen tissue sections using DQ gelatin substrate (Invitrogen, Molecular Probes, Paisley, UK).

RESULTS

In the cystic areas of CL-LCDD, elastic tissue stain revealed a quite complete and diffuse loss of elastic fibres. This process involved the alveolar walls as well as the small airways and vessels (fig. 1b and c). In the same way, a decrease of type IV collagen (fig. 1d) and collagen fibres (fig. 1e; table 2) was observed to a lesser degree. Unlike elastic fibres, collagen degradation had a heterogeneous distribution from one cystic zone to another. Macrophages, especially giant cells surrounding the deposits, expressed MMP-9 (fig. 2a), MMP-2 (fig. 2b), MMP-1 and MMP-12. Membrane positivities were also found for MMP-14 (fig. 2c). TIMP-2 and TIMP-1 were not detected (table 3). *In situ* zymography revealed a strong fluorescence with the same location as κ light chain deposits (fig. 1f).

As in CL-LCDD, the current authors showed that the majority of elastic fibres located just beneath LCH infiltrates and in the areas of LAM cell accumulation were lacking. However, remodelling of type-IV and fibrillar collagens was distinct from CL-LCDD. In LCH, almost all basement membranes had disappeared in the cyst wall. Fibrosis was absent or present

TABLE 1 Immunohistochemical reagents

Antigen	Vendor	Clone	Dilution	Comments
Type IV collagen	DAKO[#]	M0785	1:40	Major component of basement membrane
MMP-1	Calbiochem[¶]	IML35	1:60	Fibrillar collagens proteolysis [17]
MMP-2	Chemincon[+]	MAB 134431	1:30	Elastic fibres [17], type IV collagen proteolysis
MMP-9	Calbiochem[¶]	IM37	1:20	Elastic fibres [17], type IV collagen proteolysis
MMP-12	R&D Systems[§]	MAB 917	1:40	Elastic fibres proteolysis [18]
MMP-14	R&D Systems[§]	AF918	1:50	MMP-2 membrane activation [19]
TIMP-1	Calbiochem[¶]	IM63	1:30	Tissue MMP inhibition
TIMP-2	R&D Systems[§]	MAB 9711	1:40	Tissue MMP inhibition and implication in MMP-2 activation

MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase. [#]: DAKO, Glostrup, Denmark; [¶]: an affiliate of Merck, Darmstadt, Germany; [+]: Chemincon, Temecula, CA, USA; [§]: R&D Systems, Minneapolis, MN, USA.

according to the early or late stage of the lesion, respectively. In LAM, each smooth muscle cell located in the cyst wall was lined by its own basement membrane material and collagen fibres were very sparse (table 2). MMPs expressed in LCH were similar to those found in CL-LCDD but the labelled cells were different. In contrast, few MMPs (MMP-1 and MMP-14) were detected in LAM cells (table 3).

DISCUSSION

The major finding of the present study is the demonstration of extensive degradation of the elastic pulmonary network in CL-LCDD associated with high expression of numerous MMPs by macrophagic giant cells.

The literature on monoclonal Ig deposits and ECM changes is limited. Monoclonal Ig may induce ECM expansion, as demonstrated by the occurrence of the nodular glomerulosclerosis in systemic LCDD [21]. Alternatively, fragmentation and loss of elastic fibres was reported in a patient affected by systemic LCDD with unusual lung involvement [21]. In the same way, the current authors found reports of three patients with multiple myeloma complicated by skin lesion combining light chain amyloidosis and elastolysis [22, 23]. In the present study, ECM investigation in CL-LCDD showed a widespread and quite complete degradation of the elastic network involving alveoli, small airways and vessels. Loss of the fibrillar and basement membrane collagens was also observed, but the intensity and extent of the lesions were less severe. Like CL-LCDD, LCH and LAM were characterised by the lack of elastic fibres in cystic areas, but remodelling of type-IV and fibrillar collagens was different. The same ECM modifications have been previously demonstrated [14, 15, 24]. Moreover, degraded elastic fibres have also been shown by electron microscopy [14, 15]. Since elastic fibres allow distensibility of the lung during inspiration and elastic recoil during expiration, the current authors propose that decrease in the number of elastic fibres may account for the cyst development process.

TABLE 2 Extracellular matrix (ECM) changes by comparison with normal lung tissue in cystic lung light chain deposition disease (CL-LCDD), Langerhans' cell histiocytosis (LCH) and lymphangioleiomyomatosis (LAM) cystic walls

	CL-LCDD	LCH	LAM
Subjects n	4	3	3
Elastic fibre	↓↓↓	↓↓↓	↓↓↓
Fibrillar collagens	↓	↑↓	↓↓↓
Type-IV collagen	↓↓	↓↓↓	↑↑

↑: increase; ↓: decrease; ↑↓: variable according to the stage of the lesion. The intensity of ECM change was graded on a scale of 1 to 3 arrows (1: mild; 2: moderate; 3: severe).

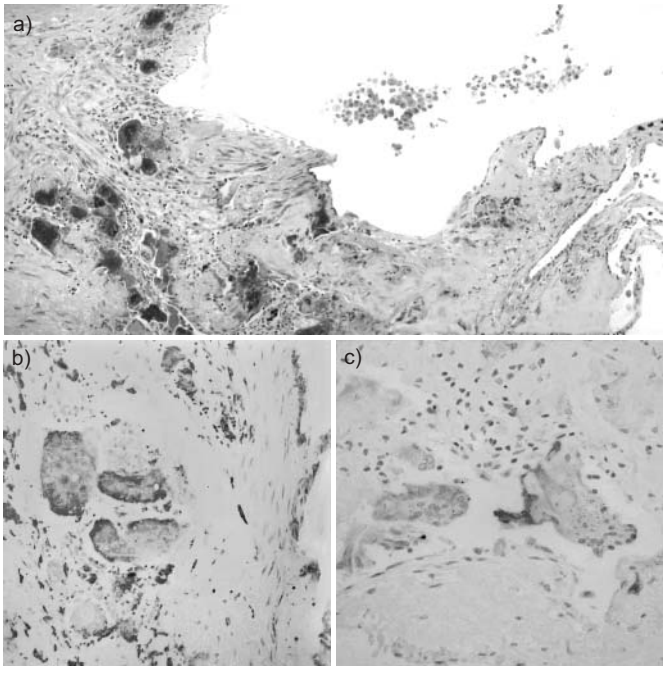

FIGURE 2. Matrix metalloproteinase (MMP) immunostainings in cystic lung light chain deposition disease. The macrophagic giant cells adjacent to monoclonal κ light chain deposits strongly express MMP-9 (a), contrary to the macrophages accumulated in the alveoli. The macrophagic giant cells also express MMP-2 (b) and its activator, MMP-14 (c).

TABLE 3 Matrix metalloproteinase (MMP) expression profile in cystic lung light chain deposition disease (CL-LCDD), Langerhans' cell histiocytosis (LCH) and lymphangioleiomyomatosis (LAM)

Labelled cells	CL-LCDD	LCH				LAM
	Macrophagic giant cells	Langerhans cells	Macrophages	Eosinophils	Fibroblasts	LAM cells
Subjects n	4	3	3	3	3	3
MMP-1	+	-	+	-	+	+
MMP-2	+	+	+	-	+	-
MMP-9	+	+	+	+	-	-
MMP-12	+	-	+	-	-	-
MMP-14	+	+	+	-	-	+
TIMP-1	-	-	-	-	-	-
TIMP-2	-	-	+	-	-	-

TIMP: tissue inhibitor of metalloproteinase; +: present; -: absent

ECM components are the target of MMPs that are principally produced by stromal cells. In systemic multivisceral LCDD with renal involvement, MMPs play a pivotal role in glomerular remodelling [21]. Moreover, as macrophagic giant cells are known to be able to cleave elastin *via* MMP production [25, 26], it was hypothesised that these cells may be implicated in the mechanism of cyst formation in CL-LCDD. The fact that macrophagic giant cells expressed numerous MMPs able to degrade elastic fibres (MMP-2, MMP-9 and MMP-12), fibrillar collagens (MMP-1) and type-IV collagen (MMP-2 and MMP-9) argues for this hypothesis. MMP-14, the principal activator of MMP-2, was also detected, while TIMP-1 and TIMP-2 were not expressed. *In situ* zymography confirmed a strong MMP-2 and MMP-9 activity. The same MMP expression profile was found in LCH. The striking difference between the two disorders was based on the involved cells. The role of MMPs in LCH has been previously assessed in only one study that disclosed a similar MMP expression profile [27]. These enzymatic patterns indicate that the reduction of pulmonary elastic fibres in CL-LCDD and LCH probably share a common pathogenesis that may be related to the activity of MMP, but the cells implicated are different. Contrary to CL-LCDD and LCH, very few MMPs (MMP-1 and MMP-14) were expressed in LAM. Their role, which has been addressed by occasional studies [16, 28, 29], remains controversial.

In conclusion, the present study indicates that pulmonary elastolysis represents the common denominator between cystic lung light chain deposition disease and other cystic lung disorders. Moreover, the expression of several elastolytic matrix metalloproteinases, added to the gelatinolytic activity observed in cystic lung light chain deposition disease, provides strong support for the role of matrix metalloproteinases in the degradation of elastic fibres. Therefore, the current authors postulated that the steps for pulmonary cyst formation in cystic lung light chain deposition disease may be the following: 1) recruitment of macrophages around light chain deposits; 2) production of the matrix metalloproteinases responsible for elastin destruction by these cells, thereby explaining the fragility of the lung parenchyma; and 3) enlargement and rupture of the alveoli and bronchioles.

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