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ORIGINAL ARTICLE

Expression and arrangement of extracellular matrix proteins in the lungs of mice infected with *Paracoccidioides brasiliensis* conidia

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Summary

Extracellular matrix (ECM) proteins are important modulators of migration, differentiation and proliferation for the various cell types present in the lungs; they influence the immune response as well as participate in the adherence of several fungi including *Paracoccidioides brasiliensis*. The expression, deposition and arrangement of ECM proteins such as laminin, fibronectin, fibrinogen, collagen and proteoglycans in the lungs of mice infected with *P. brasiliensis* conidia has been evaluated in this study, together with the elastic fibre system. Lungs of BALB/c mice infected with *P. brasiliensis* conidia were analysed for the different ECM proteins by histological and immunohistochemical procedures at different times of infection. In addition, laser scanning confocal microscopy and scanning electron microscopy were used. During the early periods, the lungs of infected animals showed an inflammatory infiltrate composed mainly of polymorphonuclear neutrophils (PMNs) and macrophages, while during the later periods, mice presented a chronic inflammatory response with granuloma formation. Re-arrangement and increased expression of all ECM proteins tested were observed throughout all studied periods, especially during the occurrence of inflammatory infiltration and formation of the granuloma. The elastic fibre system showed an elastolysis process in all experiments. In conclusion, this study provides new details of pulmonary ECM distribution during the course of paracoccidioidomycosis.

Keywords
collagen, extracellular matrix proteins, fibrinogen, fibronectin, laminin, *Paracoccidioides brasiliensis*

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Extracellular matrix (ECM) proteins are considered to be essential constituents of tissues' support structures. They are also important modulators of migration, differentiation, proliferation and function of the various cell types present into the lungs. Additionally, these ECM compounds influence directly or indirectly the immune responses including the

inflammatory process and the repair of pulmonary tissues. After lung injury, e.g. in asthma, there is evidence of epithelial cell damage and infiltration of inflammatory cells, together with hypertrophy and hyperplasia of goblet cells, submucosal glands and airway smooth muscle. Additionally, there is increased deposition of extracellular matrix molecules including different types of collagens (I, III and V) (Roche *et al.* 1989; Chu *et al.* 1998), fibronectin (Roche *et al.* 1989), and proteoglycans (Huang *et al.* 1999) in the sub-epithelial *lamina reticularis*. In addition, of the ECM components, the elastic system plays an important role in maintaining the patency of the airways as well as the elastic recoil of the lung. Therefore, elastosis might be an important event in understanding the alterations in pulmonary function (Mercer & Crapo 1990; Rozin *et al.* 2005).

The interaction of ECM proteins with several infectious agents has been reported. Expression, arrangement and deposition of ECM proteins induced by different micro-organisms such as *Mycobacterium avium* (Sato *et al.* 2003), *Mycoplasma pneumoniae* (Chu *et al.* 2005), *Schistosoma mansoni* (Lenzi *et al.* 1999), *Toxoplasma gondii* and *Trypanosoma cruzi* (Silva *et al.* 1999) have been previously noticed. ECM proteins have been shown to serve as adherence substrates for several pathogens (Silva-Filho *et al.* 1988; Furtado *et al.* 1992; Li *et al.* 1995; Gaur *et al.* 1999; Wasylnka & Moore 2000). Of particular interest in this area is the identification of ECM-binding proteins on the surface of certain fungi of clinical importance such as *Candida albicans* (López-Ribot *et al.* 1996; Gaur *et al.* 1999), *Aspergillus fumigatus* (Wasylnka & Moore 2000), *Histoplasma capsulatum* (McMahon *et al.* 1995), *Cryptococcus neoformans* (Rodrigues *et al.* 2003), *Pneumocystis carinii* (Narasimhan *et al.* 1994), *Penicillium marneffei* (Hamilton *et al.* 1999) and *Paracoccidioides brasiliensis* (González *et al.* 2005).

Paracoccidioides brasiliensis is the aetiological agent of paracoccidioidomycosis (PCM), an endemic, mycosis of importance in Latin America, especially in Brazil, Colombia and Venezuela (Restrepo & Tobon 2005). Usually, it is a chronic, progressive illness that involves several organs and systems, mainly the lungs, taken to be the primary site of infection (Restrepo & Tobon 2005), where its progression is often accompanied by fibrotic sequelae, a complication that may severely hamper respiratory functions (Tobón *et al.* 2003).

After intraperitoneal inoculation with *P. brasiliensis,* yeast cells in different experimental animals (albino rats, nu/nu and nu/+ and B10A mice) lesions develop with two patterns of ECM distribution being observed: interstitial characterized by the presence of laminin, collagen fibronectin, proteoglycans and minimal amount or absence of tenascin, and proteins directly bound to the *P. brasiliensis* yeast surface and/or cytoplasma. The binding pattern is predominantly expressed by the daughter cells or buds and characterized by the presence of laminin and fibronectin (Mendes-Giannini *et al.* 2000). Despite of these above observations, the importance of tissue ECM deposition in the pathogenesis of PCM is poorly understood.

The aim of this work was to determine the expression, deposition and arrangement of ECM proteins and also of the elastic fibres system in the lungs of mice infected with *P. brasiliensis* conidia.

Materials and methods

Animals

Isogenic 6-week-old BALB/c male mice, obtained from the breeding colony of the Corporación para Investigaciones Biológicas (CIB), Medellín-Colombia, were used in all experiments, and were kept and fed under the conditions previously indicated (Restrepo *et al.* 1992). Mice were supplied with sterilized commercial food pellets, sterilized bedding and fresh acidified water; their care took into consideration the recommendations given by the Colombian Government (Law 84 of 1983, Rs No. 8430 of 1993) and the regulations of the European Communities and Canadian Council of Animal Care (1998).

Fungus culture and conidia production

Paracoccidioides brasiliensis isolate ATCC 60855, previously known to sporulate freely on special media, was employed (Restrepo *et al.* 1986). The techniques used to grow the mycelial form, collect and dislodge conidia have been reported previously (Restrepo *et al.* 1986). Briefly, the stock mycelial culture was grown in a liquid synthetic medium, the modified McVeigh-Morton broth at 18 °C (± 4 °C) with shaking. Growth was homogenized and portions were used to inoculate agar plates; the latter were incubated at 18 °C (± 4 °C) for 3 months. After this time, sterile physiological saline containing 0.01% Tween-20, plus 100 U penicillin and 100 μg/ml streptomycin, was used to flood the culture surface. Growth was removed with a bacteriological loop and the resulting suspension pipetted into an Erlenmeyer flask containing glass beads. This was then shaken in a reciprocating shaker at 250 rpm for 45 min. The homogeneous suspension was filtered through a syringe packed with sterile glass wool (Pyrex fibre glass, 8 microns, Corning glasswork, Corning, NY, USA). The filtrate was collected in a polycarbonate centrifuge tube and centrifuged for 30 min

at 1300 × *g*; the pelleted conidia were washed, counted with a haemacytometer, and their viability assessed by the ethidium bromide-fluorescein diacetate technique (Calich *et al.* 1978). For the experiments, only inocula with a conidial viability > 90% were used.

Paracoccidioides brasiliensis *experimental infection*

Animals were anaesthetized by intramuscular injection of 50 μl of a solution that contained a mixture of ketamine hydrocloride (100 mg) (Park-Davies & Co., Bogotá, Colombia) and xylazine (20 mg) (Bayer, SA, Brazil). When deep anaesthesia was obtained, 4×10^6 conidia suspended in a 60 μl inoculum divided in two portions were instilled intranasally within a 10 min period. The abdomen was compressed and droplets were deposited on the nares; when the pressure was released, the mouse inhaled deeply. Control mice received an intranasal inoculum of 60 μl of PBS. The mice were sacrificed after different periods postchallenge [0 (2 h postinoculation), 1, 2, 3, 4 days, and 1, 2, 4, 8 and 12 weeks). At each period, five mice from each experimental group, as well as five non-infected control animals, were sacrificed by the intraperitoneal injection of 1.0 ml of 2.5% sodium penthotal (Abbott Laboratories, Chicago, IL, USA). Different mouse groups were used for histology and immunohistology studies.

Antibodies

The following polyclonal antibodies were used in this study: rabbit immunoglobulin to human fibrinogen (DAKO, Carpinteria, CA, USA; Cat. # A080), rabbit immunoglobulin to human fibronectin (DAKO, Cat. # A245), monoclonal mouse antibody to human laminin (DAKO, Cat. # 4C7), FITC-immunoglobulin anti-rabbit IgG developed in goat (DAKO, Cat. # F0205), FITC-anti-mouse IgG, developed in rabbit (DAKO, Cat. # F261), and Alexa Fluor 488 Rabbit anti-goat IgG (Molecular Probes; Eugene OR, USA; Cat. # A110778).

Histology

Upon sacrifice, the animals' thoracic cavity was opened and the right auricle sectioned; 10 ml of PBS were then injected directly into the heart in order to inflate the lungs and withdraw any remaining blood. Lungs were excised, frozen and placed immediately into a solution of Optimal Cutting Temperature (OCT) (embedding medium for frozen tissue specimens; Tissue Tek Miles Inc., Diagnostics Division, Elkhart, IN, USA), and subsequently stored at −70 °C. For histology preparation, lungs were excised as described previously, except that they were fixed in 10% neutral formaldehyde in PBS, embedded in paraffin, cut in 5 μm thick sections and stained with haematoxylin and eosin (H&E), Lennert's Giemsa, and Sirius Red to determine the type and intensity of the inflammatory response; Silver methenamine (Grocott) was used to detect the mycotic structures (conidia and yeast cells), periodic acid-schiff (PAS) reaction to detect neutral glycoproteins, PAS-Alcian blue (PAS-AB), pH 1.0 and 2.5, to demonstrate high and low sulfated proteoglycans, and Weigert's resorcin-fuschin method following prior oxidation, to detect elastic fibres (this method allows the selective identification of the three types of elastic system fibres namely oxytalan, elaunin and fully developed elastic fibres). For bright-field microscopy, Masson's trichrome and Gomori's silver reticulin (plus polarization microscopy) were used for identification of collagen I and III. In addition, thick histological sections (20–30 μm) were stained with phosphomolybdic acid-picrosirius red (PMA-PSR) for three-dimensional study of collagenous fibres by confocal laser scanning microscopic (CLSM), finally, deparaffined sections were desiccated in an incubator at 37 °C for 1 h and analysed by scanning electron microscopy (SEM), low vacuum mode (Model 435-VP, Leo, Cambridge, UK).

Immunohistochemistry

In order to investigate the expression of ECM proteins during the course of infection, lungs that had been frozen were embedded in OCT and 5 μm sections were cut with a cryostat. If staining was not performed immediately, slides were stored at −20 °C in a sealed slide box. For staining, slides were fixed in acetone for 15 min, and then rehydrated in PBS, pH 7.4. To prevent non-specific interactions of the antibodies, sections were treated with fetal bovine serum (FBS) 8%, bovine serum albumin (BSA) 2.5%, and skim milk 2.0% in PBS pH 7.4, for 30 min at room temperature in a humid chamber. Sections were then incubated with the antibody diluted in BSA 1% in PBS pH 7.4 for 60 min at 37 °C in humid chamber. All antibodies were used at previously determined optimal concentrations. Slides were washed three times in PBS pH 7.4, counterstained with Evan's blue, and mounted with anti-feeding glycerin (PBS pH 7.6 plus 100 mg of phenylendiamine and glycerol; Sigma Chemical Co., St Louis, MO, USA) and stored in humid chambers at 4 °C. All sections were examined in a photomicroscopic (Carl Zeiss III) and laser microscopic scanning (LSM-410 inverted microscope; Zeiss, Jena, Germany).

Tissue sections were evaluated blinded by a pathologist using a semi-quantitative approach. A score of + to +++ was

given according to the degree of inflammation, fungal infiltration as well as to ECM expression with (+++) for strong, (++) for moderate, (+) for weak, (±) for very weak and (−) for no reaction (Silva *et al.* 1999).

Results

Inflammatory lung responses induced by Paracoccidioides brasiliensis *conidia*

Mice inoculated intranasally with PBS presented no abnormalities in their lungs irrespective of the time of the experiment (Figure 1a). By contrast, during the initial (first 4 days) postchallenge periods mice infected with *P. brasiliensis* conidia developed a bronchopneumonic acute type response, characterized by an inflammatory infiltrate composed mainly of neutrophils and macrophages located inside the alveolar spaces and surrounding the peribronchial vessels (Figure 1b,c). On day 7, in the infected mice, lung infiltration was similar to that on day 4, but neutrophils were not as abundant. In the later periods postinfection (weeks 4–12), mice presented a chronic inflammatory response with granuloma formation and the infiltrates were constituted mainly of mononuclear cells (lympho-histio-plasmocytic cells and Langhans giant cells), especially at the perivascular level (Figure 1d). Granulomas were compact at early stages, but they became loose as infection progressed.

Eosinophils and lymphocytes were observed mainly during the first 3 days postinfection, with preference at perivascular level.

Paracoccidioides brasiliensis propagules could be observed in the tissues all throughout the study. Conidia were observed during first 3 days postinfection and yeast cells from day 2. Yeast cells were numerous, in active multiplication especially during the weeks 4–12.

Expression and deposition of ECM proteins in lungs of mice infected with P. brasiliensis *conidia*

Arrangement of the ECM proteins started with the deposition of fibrin-like material as early as 2 days, during the inflammatory period and throughout the study period (Figure 1e–h).

We observed a normal expression and deposition of fibronectin and fibrinogen, but not of laminin, in the lungs of animals inoculated with PBS (Figure 2a,e,i). In contrast, in infected animals, the lungs experienced reorganization with increased deposition of the three ECM proteins tested, during the inflammatory process and throughout the observations period except in the first week, where there was a decrease in these molecules. In addition, later on during infection (weeks 4–12) these proteins surrounded preferentially the granuloma structures, and in some occasions were also observed inside the granuloma (Figure 2).

The reticulin fibres underwent fragmentation during the first 3 days postinfection but increased after day 4, indicating the beginning of the fibrotic process (Figure 3a–d). These reticulin fibres were also observed in later periods, especially surrounding the granuloma (Figure 3c,d).

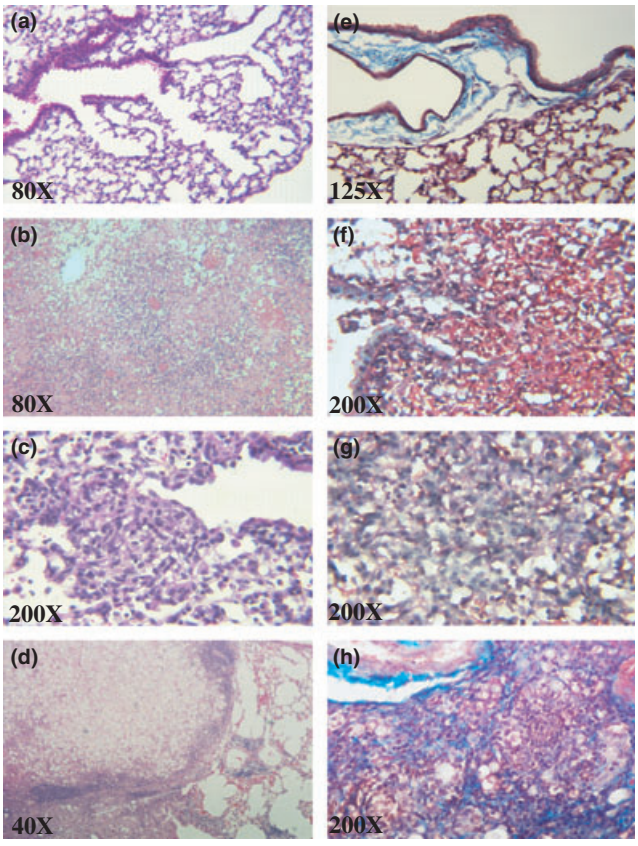

Figure 1 Histological description of lungs in mice inoculated with 4×10^6 *Paracoccidioides brasiliensis* conidia. H&E-stained sections of infected mouse lungs at: day 0 (mice killed 2 h after inoculation): no inflammatory infiltrates (a); day 2 postinfection, increase in neutrophils within alveolar spaces and congestive reaction with oedema and haemorrhage (b); day 4 postinfection, predominantly macrophage accumulation (c); in week 12, structurally arranged granulomas with two zones, one central containing the fungi, and peripheral constituted by epithelioid and lympho-histio-plasmocytic cells (d). Masson trichromic-stained sections of infected mice at day 0, normal deposition of collagen fibres (in blue colour), especially at the peribronchial level (e); deposition of fibrin-like material as early at days-2 (f) and -4 (g) postinfection; in week 12, thick type I collagen fibres surrounding and inside the granuloma (h).

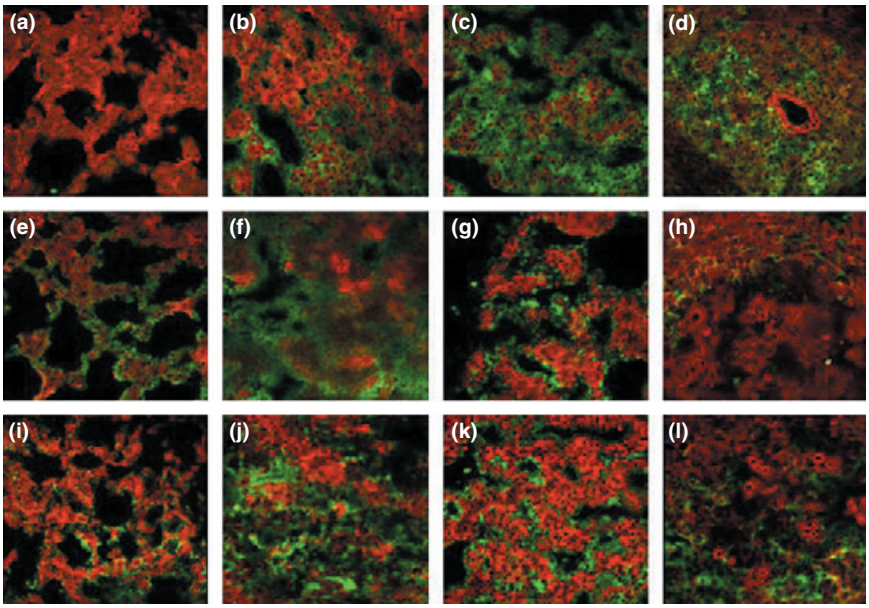

Figure 2 IF detection of ECM protein expression in the lungs of mice inoculated with PBS or *Paracoccidioides brasiliensis* conidia. Frozen sections were treated with monoclonal antibody against human laminin (a–d) and polyclonal antibodies against fibronectin (e–h) and fibrinogen (i–l). At day 0 (2 h postinoculation with PBS), lower intensity or undetected laminin (a) and normal levels of fibronectin (e) or fibrinogen (i) were observed in these tissues. At days-2 (b, f, j) and -4 (c, g, k) postinfection abnormal arrangement and increased expression of laminin, fibronectin and fibrinogen were observed. In week 12 (d, h, l) these ECM proteins were observed surrounding and inside the granulomas. Fluorescence is observed in green with the red corresponding to tissue stained with Evan's blue. Magnification 40 ×.

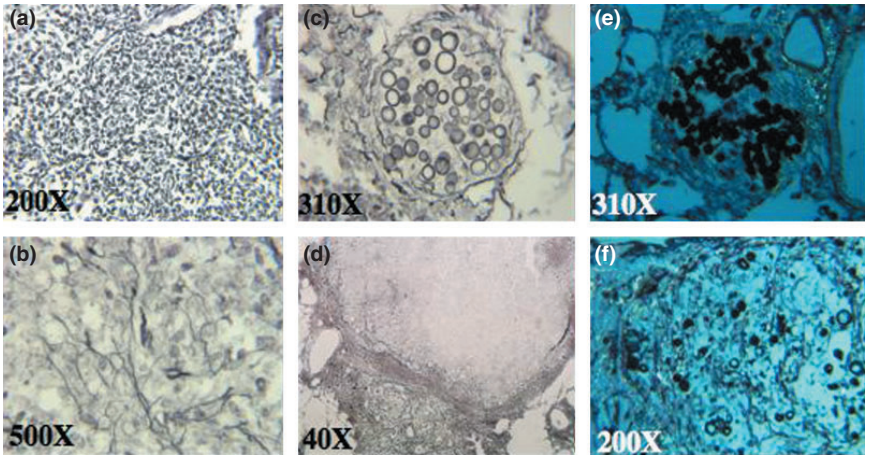

Figure 3 Histological analysis of reticulin fibres in lungs of mice inoculated with 4×10^6 *Paracoccidioides brasiliensis* conidia. Sections of infected mice lungs were stained with Gomori's silver reticulin. Decreased numbers of reticular fibres at day 2 (a). At day 4 postinfection, increase of reticular fibres, indicating the beginning of the fibrotic process (b). On weeks-2 (c) and -12 (d), reticulin fibres were observed in lesser amounts, especially surrounding the granuloma structures. Figures e and f show the presence of type III collagen fibres using polarization microscopy (all brightly birefringent structures, shining against dark background, corresponding to collagen molecules).

Using bright-field microscopy we observed thin fibres corresponding to collagen type III both early during the inflammatory process and on day 7 (Figure 3e,f).

Analysis by CLSM and using PMA-PSR stain in non-infected animals revealed a normal distribution of collagen fibres in the lungs (Figure 4a), while in the infected animals

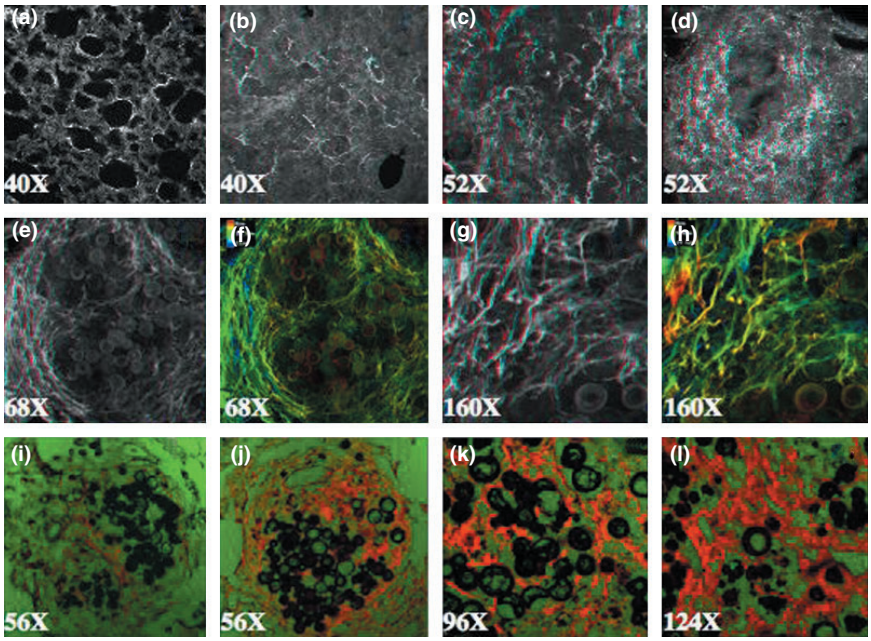

Figure 4 Determination of collagen expression in lungs of mice infected with 4×10^6 *Paracoccidioides brasiliensis* conidia. Different lungs' sections (5–10 and 20–30 μm) stained with PMA-PSR (a–h) or Grocott-PMA-PSR (i–l) all analysed by CLSM. At day 0 postinfection (mice killed 2 h after inoculation), normal expression of type I collagen were observed (a). At days 2 (b) and 4 (c) postinfection abnormal arrangement of collagen fibres. In week 12 (d) postinfection thick collagen fibres forming a honeycomb or surrounding the granuloma. Figures d–h, lung granulomas originated from 3-D, CLMS reconstructions. Figures e and g: arrangement of collagen fibres in week 4 postinfection. Figures f and h correspond to previous figures (e and g) in colour codes. Sequential increase and thickness of collagen fibres are observed in red colour and the fungi in black, at different weeks postinfection, 2nd (i), 4th (j), 8th (k) and 12th (l).

collagen fibres fragmented as did the reticulin fibres during the first days postinfection, with their increased being noticed on later periods (Figure 4b–d).

On weeks 4–12, collagen fibres appear to be formed of abundant collagen type I and in lesser amounts, of collagen type III. These collagen fibres were observed both surrounding and inside the granuloma (Figure 4).

The three-dimensional tissue architecture of the lungs, as observed by CLSM and SEM, showed ECM fibres initially arranged in a disorganized pattern; however, during the later periods postinfection and especially in and around the granuloma structures, a compact and concentric arrangement of these fibres was observed (Figures 4 and 5). The initial granuloma structure was characterized by collagen residues and by anchorage points (or fibre radiation centres).

In addition, the low sulphated proteoglycans increased during the inflammatory process including final observation periods (Figure 6a–d). These data are summarized in Table 1.

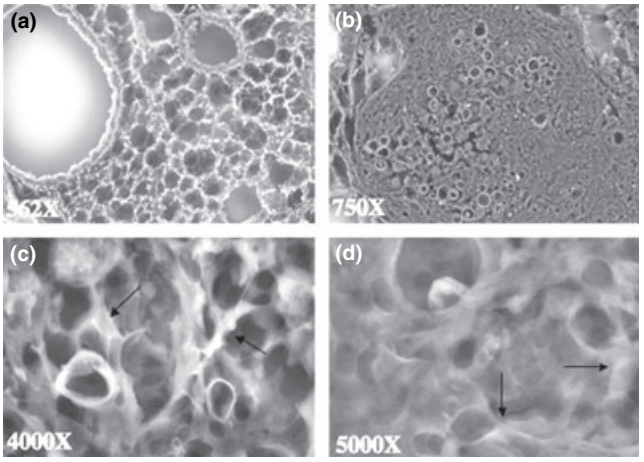

Figure 5 Thick histological sections (20–30 μm) of mouse lungs inoculated with 4×10^6 *Paracoccidioides brasiliensis* conidia or PBS were analysed using SEM. Panoramic view of a normal lung at day 0 inoculated with PBS (a). Granuloma formation on week 4, the fungi is observed within an amorphous matrix (b). Arrows indicate fibre anchorage points or fibre radiation centres seen on weeks 8 and 12 (c, d) respectively.

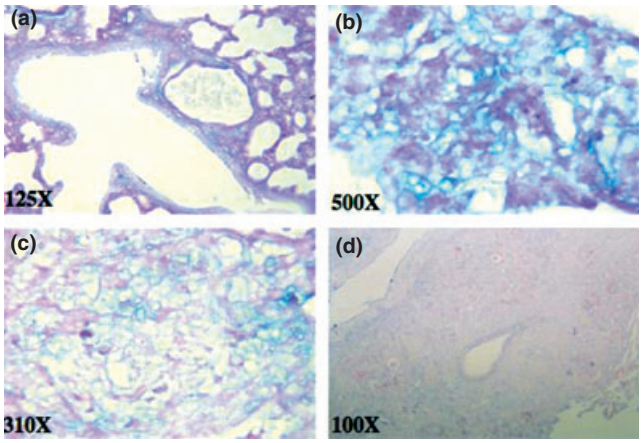

Figure 6 Histological analysis of proteoglycans in lungs of mice inoculated with 4×10^6 *Paracoccidioides brasiliensis* conidia. PAS-AB stained sections of infected mice at day 0, normal deposition of proteoglycans, especially at peribronchial and periarterial level (a); increased levels of proteoglycans at days 2 (b) and 4 (c) and in week 12 (d), within the inflammatory infiltrate and the granuloma.

Table 1 Presence of inflammatory infiltrates and expression of ECM components in lungs of BALB/c mice infected with *Paracoccidioides brasiliensis* conidia

	Experimental periods (postinfection)						
	Days			Weeks			
Analysed parameter	Uninfected	2	4	1	4	8	12
Inflammatory infiltrates	–	+++	+++	++	++	++	+++
Granulomas	–	–	–	+	++	+++	+++
Laminin	–	+	++	+	++	+++	+++
Fibronectin	+	++	++	+	++	++	++
Fibrinogen	+	++	++	+	++	++	++
Collagen type III	±	±	+	+	+	+	+
Collagen type I	+	±	+	+	++	++	+++
Proteoglycans	+	+++	++	+	++	++	++
Elastic fibers	++	+	±	±	±	±	±

The occurrence/intensity of the analysed parameters was scored as follows: +++, strong; ++, moderate; +, weak; ±, very weak; and –, no reaction.

Elastic system fibres

In control animals, the distribution of the elastic fibre system in the lungs was normal (Figure 7a). However, when the animals were infected with *P. brasiliensis* conidia, the elastic fibres in their lungs showed important changes especially since day 4 postinfection when sparse and fragmented bundles of elastic fibres were noticed, indicating an elastolysis process (Figure 7b,c). Later (weeks 4–12), these elements had diminished and could not be seen inside the granulomas (Figure 7d, Table 1).

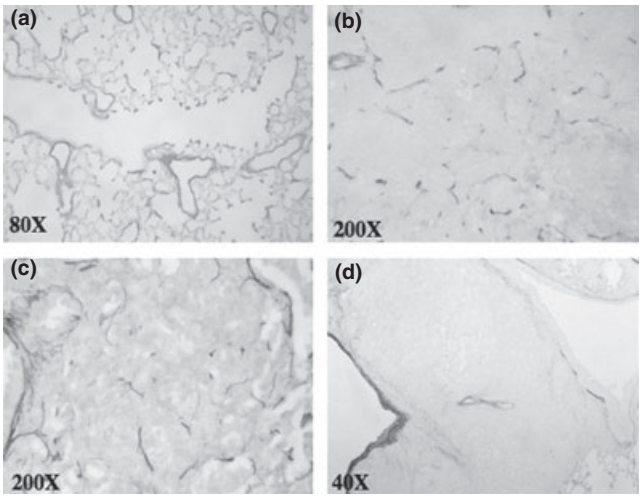

Figure 7 Histological description of lungs in mice inoculated with 4×10^6 *Paracoccidioides brasiliensis* conidia. Lungs sections were stained with Weigter's resorcin-fuschin with prior oxidation for elastic fibre identification. At day 0 (mice killed 2 h after inoculation) normal arrangement and deposition of elastic fibres (a); elastolysis at days 2 (b) and 4 (c) and on week 12 (d) postinfection, an elastolysis process is observed (b–d).

Discussion

In the present study, we investigated the pattern, expression, deposition and arrangement of ECM components in the lungs of mice during experimental infection with *P. brasiliensis* conidia. In addition, we observed the sequential histopathological changes that occurred as a result of fungal infection. The progressive histopathological abnormalities observed in BALB/c mice were attributed to the infection and confirmed previous findings concerning the pulmonary tissue responses induced by the fungal infection (Restrepo *et al.* 1992; Franco *et al.* 1998; Cock *et al.* 2000). Initially, an afflux of PMNs was noticed, which then changed towards a lympho-histo-plasmocytic infiltrate ending up in granuloma formation.

Deposition of ECM molecules increased gradually, especially during the granulomatous stages. This process started with presence of fibrin-like material during the acute inflammatory process.

Initially, during the first days postinfection, we observed that the different ECM proteins tested (fibronectin, laminin and fibrinogen) had increased, concomitantly with the marked afflux of pro-inflammatory cells, suggesting a

possible participation of these ECM compounds in the migration of inflammatory cells into the lungs during *P. brasiliensis* infection. Concerning collagen, we detected destruction of collagen fibres during the first 3 days postinfection; thereafter, on day 4, reticulin fibres corresponding to collagen type III also showed an increase. In first week, we observed a decrease in almost all parameters analysed; this behaviour has been observed previously by other investigators (Cock *et al.* 2000), who have noticed that in this early period, both the inflammatory process and the fungal burden decrease indicating that the infection is apparently under control. However, such infectious process reactivated later on from week 2 postchallenge (Cock *et al.* 2000).

During the evolution of infection (weeks 2–12), we recorded development of granulomas which displayed a pattern of ECM composed of laminin, fibronectin, fibrinogen, collagen type I, and a lesser amount, also of collagen type III and proteoglycans. The granulomas were structurally arranged in two zones: a central zone containing fungi, and peripheral zone formed by epithelioid and mononuclear cells, as previously described in other animal models (Kerr *et al.* 1988; Mendes-Giannini *et al.* 2000). The majority of ECM proteins studied were found surrounding the granuloma, but sporadically they were also seen inside these structures.

In later periods (weeks 4–12) postinfection, especially at time of granuloma formation, the minimal 'unit' was formed by a fibrilar cocoon of reticular fibres that enclosed an individual yeast, or a group composed of a large parent cell plus one of various peripheral daughter cells or buds. The same pattern had already been observed independent of the type of mice, lesion, or fungus strain (Mendes-Giannini *et al.* 2000).

CLMS and SEM methods allowed to construct a three-dimensional image of the lung's architecture, especially of the granuloma, which displayed the presence of ECM fibres that were initially arranged in a disorganized fashion, but that finally evolved towards a compact, concentric arrangement. We observed that this arrangement of ECM fibres, especially collagen fibres originated from an anchorage point. This anchorage point had been previously described by Lenzi *et al.* (1999), who denominated this structure as a 'fibre radiation centre' in a schistosomiasis model and suggested that such anchorage points served to provide increased tissue integrity, an efficient distribution of soluble compounds (growth factors and cytokines) and haptotactic background to the cells (Lenzi *et al.* 1999).

Paracoccidioides brasiliensis can disseminate to several organs. However, the manner in which the fungus gains access to them is unclear, but it may involve migration of infected macrophages or dendritic cells through the lymphatic system (Restrepo & Tobon 2005). Degradation of ECM proteins may also play a role in this dissemination process (Puccia *et al.* 1998). Furthermore, migration of macrophages and other leucocytes to sites infected with *P. brasiliensis*, as well as the expression of a granulomatous response (Cock *et al.* 2000; González *et al.* 2003), require degradation of ECM proteins, as well as of the basement membrane. Likewise, it has also been demonstrated that *P. brasiliensis* is able to cleave type IV collagen, fibronectin, laminin and proteoglycans through an exocellular serine-thiol proteinase (Puccia *et al.* 1998).

One of the earliest events in the inflammatory process is the increased tendency of circulating leucocytes to adhere to the endothelial cell's luminal surfaces. Leucocyte migration, antigen recognition, activation and differentiation are events affected by the ECM molecules. Moreover, the ability of leucocytes to recognize ECM components may change as they migrate into tissue, presumably influenced by signals from the microenviroment (Romanic *et al.* 1997). It has also been shown that the influx of inflammatory cells into the target tissue is associated with remodelling of the ECM (Kieseier *et al.* 1998).

Fibrin and other ECM proteins are believed to play a role in the formation and maintenance of granuloma (Izaki *et al.* 1979; Marshall *et al.* 1996) with fibrin(ogen) reportedly stimulating the expression by macrophages/monocytes of tumor necrosis factor-alpha (TNF-α) and interleukin 1-beta (IL-1β) (Kajikawa *et al.* 1986; Perez & Roman 1995). Sato *et al.* (2003) studied the importance of the fibrinolytic system during *Mycobacterium* infection demonstrating that plasmin(ogen) plays a role in the turnover of ECM proteins within the granulomas albeit its effect in the early dissemination of *M. avium* from the lungs is limited. Thus, plasmin(ogen) functions in limiting progressive fibrosis in the granuloma during chronic mycobacterial infection (Sato *et al.* 2003). In this study, we found an increased expression of fibrinogen during all the periods studied. We have also demonstrated high levels of TNF-α and IL-1β during the first days postinfection as well as in late periods (Franco *et al.* 1998; González *et al.* 2003).

ECM molecules also play another important role in the host-parasite interaction related to adhesion of micro-organisms to host tissues, a crucial first step in the establishment of infection and subsequent dissemination. This type of adhesion is mediated by specific fungal cell-surface components called adhesins, which frequently bind ECM proteins (Patti & Hook 1994). Recently, we demonstrated the presence of two molecules of 19 and 32 kDa, on the surface of *P. brasiliensis*, conferring to the fungus the capacity to bind

to laminin, fibronectin and fibrinogen (González *et al.* 2005). In addition, we have produced a monoclonal antibody (MAb-2G4) against the 32-kDa protein was capable of inhibiting the adherence to these three ECM proteins, indicating that this adhesin is important in the adherence process (González *et al.* 2005).

The function of the elastic system is much less understood. Despite advances in the understanding its structural complexity, the interaction between elastin and the microfibril components of the elastin fibre system remains a matter of speculations, mainly in the face of lung remodelling and repair after injury (Rocco *et al.* 2001). The elastic fibres are composed of an amorphous component, called elastin, and a highly structured microfibrillar non-distensible component (Montes 1996). In normal alveolar septa, a subepithelial layer of elastic fibres confers considerable alveolar elasticity in normal situations (Mercer & Crapo 1990). Increased elastin destruction takes place in certain pathological conditions due to the release of powerful elastolytic proteases by inflammatory cells (Fukuda *et al.* 1990). Reactivation of elastin synthesis is observed in response to its increase destruction, but in a highly disorganized fashion with deleterious consequences to the mechanical properties of the lung, as it contribute to the loss of the normal alveolar architecture, leading to collapse and impairement of the inflammatory resolution mechanism (Raghow *et al.* 1985; Mariani *et al.* 1995). The occurrence of elastosis has been demonstrated in animal models of pulmonary fibrosis; these studies suggest that elastin gene expression is increased following injury (Raghow *et al.* 1985; Mariani *et al.* 1995). However, the deposition of elastic system fibres has been largely ignored in attempts to understand the pulmonary remodelling in PCM. In this study, we demonstrated that the elastic system fibres are impaired in the lungs of mice infected with *P. brasiliensis* conidia; this phenomenon could be induced by the cells involved in the initial inflammatory process.

In summary, this study provides new details of pulmonary ECM distribution during the course of PCM. However, more studies to evaluate ECM proteins expression using complementary methods must be done in order to understand the mechanisms related with the fibrotic process developed in patients with PCM.

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