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Pulmonary lymphoid lesions in an experimental model of collapsing glomerulopathy in rats

M.C. Ávila-Casado[1,3], I. Pérez-Torres[1], A. Aurón[1], A. Zepeda Rodríguez[3], V. Soto[1], E. Soria[1], S. Hernández[1], B. Chávez-Rentería[1,3], P. Mussali-Galante[3], M.R. Ávila-Costa[2] and T.I. Fortoul[3]

[1]Departamento de Patología Instituto Nacional de Cardiología, México City, México, [2]FES-Iztacala, Edo.de México, UNAM and [3]Departamento de Biología Celular y Tisular, Facultad de Medicina, UNAM. México City, México

Summary. The characterization of lung damage in an experimental model of collapsing glomerulopathy (CG) in rats is described. Methods: 12 rats were divided into two groups and injected intravenously (iv) with 1 mg/ saline in a final volume of 1 ml/ day in the tail vein for 5 days, with fractionated serum from control and CG subjects. Proteinuria was quantified, and the Glomerular filtration rate was calculated based on creatinine clearance (CC). Rats were sacrificed by perfusion fixation at day 5. Results: Rats injected with serum from CG patients developed proteinuria ($p<0.001$). A decrease in CC (0.68±0.19) in these rats was also observed. Glomerular tuft retraction and mesangial proliferation was observed in all rats receiving serum from the CG patients. Peribronchiolar infiltrate integrated mainly by lymphocytes, was identified in all CG rats. In some areas this infiltration disrupted the basement membrane and damaged the epithelium. No histopathological abnormalities in the kidney or lungs were found in rats receiving control serum. Conclusion: Patchy pulmonary lymphoid infiltrates were found in the CG model. Up to now there was no information about pulmonary lymphoid infiltration in CG patients. Besides fluid overload due to renal insufficiency or a nephrotic syndrome, other causes of pulmonary involvement in CG patients should be explored.

Key words: Collapsing glomerulopathy, Pulmonary lymphoid infiltrates, Follicular bronchiolitis, LIP

Introduction

Collapsing glomerulopathy (CG), a well-defined form of glomerular injury was first described in 1986 (Weiss et al., 1986). Six cases with nephrotic syndrome, progressive irreversible renal failure and characteristic histopathological changes were described by them. Histopathological changes consisted of glomerular capillary retraction or collapse and visceral epithelial cell swelling accompanied by tubular cystic dilatation and interstitial fibrosis. Clinically, patients with CG present a nephrotic syndrome with profuse proteinuria generally more than 10g/day, and a rapid course to end-stage renal failure or death due to complications in the nephrotic syndrome (Weiss et al., 1986). Epidemiological findings show a strong predominance in the African-American population, as is well known for renal diseases in general (Detwiler et al., 1996). The diagnosis of primary CG requires the lack of evidence of HIV infection since HIV-nephropathy can be morphologically and clinically indistinguishable from CG (D'Agati et al., 1989; Avila-Casado et al., 1994; Dantal et al., 1994; Detwiler et al., 1996; Valeri et al., 1996; Cushley et al., 1999). It has been suspected that collapsing glomerulopathy, focal and segmental glomerulosclerosis and HIV-nephropathy may have a similar pathophysiological mechanism of damage to the visceral epithelial cell. Fifteen to fifty five percent of the patients with focal and segmental glomerulosclerosis that undergo renal transplantation have recurrent disease (Detwiler et al., 1996; Valeri et al., 1996). Both recurrent and *de novo* CG have been described in the renal allograft with histopathological features similar to CG in a native kidney (Avila-Casado et al., 1994; Detwiler et al., 1996). Recurrence of CG can occur hours after transplantation raising the possibility that the plasma of CG patients contains one or more factors capable of inducing proteinuria due to the damage of the visceral epithelial cell that results in an increased glomerular permeability (Detwiler et al., 1996).

We have already developed a model of glomerular damage due to the injection of circulating factors of CG patients to rats (Avila-Casado et al, 1998). As part of the histopathological study of a rat model of collapsing glomerulopathy we decided to evaluate other organs in order to discover their possible participation in the disease. In this paper we report the pulmonary

Offprint requests to: Teresa I. Fortoul MD, PhD, Depto. Biología Celular y Tisular, Edificio A 3er piso, Fac. Medicina, UNAM CP 04510 Mexico City, Mexico. Fax: 525 (55) 623-2399. e-mail: fortoul@servidor.unam.mx

histopathological findings. Up to now there are no previous reports of pulmonary involvement.

Material and methods

Fractionation of serum

We used serum from 10 patients that fulfilled clinical and histopathological criteria of CG. Serum from healthy subjects from the Blood Bank, paired by age and sex, were used as control. Serum was fractionated by affinity-column chromatography with Affigel-Blue (Bio-Rad, Hercules, Calif., USA) (Dantal et al., 1994). Eluates in sodium-phosphate (10 mM NaCl 0.015M) were analyzed by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis on 10 percent polyacrylamide gradient gels under reducing conditions to calculate the molecular weight (Dantal et al., 1994). Eluates were dialyzed extensively against 0.001M phosphate-buffered saline using a cellulose filter membrane (Sigma, D-9277) at 4 °C and were concentrated (final protein concentration 25 mg/ml).

Injection of protein A eluates to rats

Twelve female Sprague-Dawley rats (250 to 300 g, Charles River, USA) were randomly distributed and placed in metabolic cages (Nalgene) with water and food *ad libitum*. They were divided into 2 groups (n=6). Group 1 received eluted proteins after Protein-A adsorption of serum from CG patients. Group 2 was given eluted proteins after Protein-A adsorption of serum from healthy subjects. Serum was administered i.v. once daily in the vein of the tail for 5 days in a concentration of 25 mg/ml in 1ml/saline, after previous sedation with ether in a narcosis chamber (Daigger).

Urine was collected every 24 h for 6 days: prior to the first injection (baseline, day 0), and 24, 48, 72, 96, and 120 h after the first injection (days 1 to 5 respectively). Proteinuria was measured using the method described by Biuret (Gormall et al., 1949). Rats were sacrificed 24 hrs after the last injection. The right kidney was perfused-fixed in situ with a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in cacodylate buffer (pH 7.4) for light and electron microscopy.

Table 1. Creatinine clearance from control and CG rats.

	UCr/ml	SCr/ml	CCr (ml/min)
Group 1	$1.2x10^{-4}±1.4x10^{-5}$	$2.0x10^{-6}±2.7x10^{-7}$	0.46±0.07*
Group 2	$1.5x10^{-4}±2.0x10^{-5}$	$1.7x10^{-6}±1.7x10^{-7}$	0.99±0.45

*: p≤ 0.05 Fractionated serum from CG patients vs Fractionated serum from healthy subjects; UCr/ml: Urinary Creatinine per ml; SCr/ml: Serum Creatinine per ml; CCr: Creatinine Clearance.

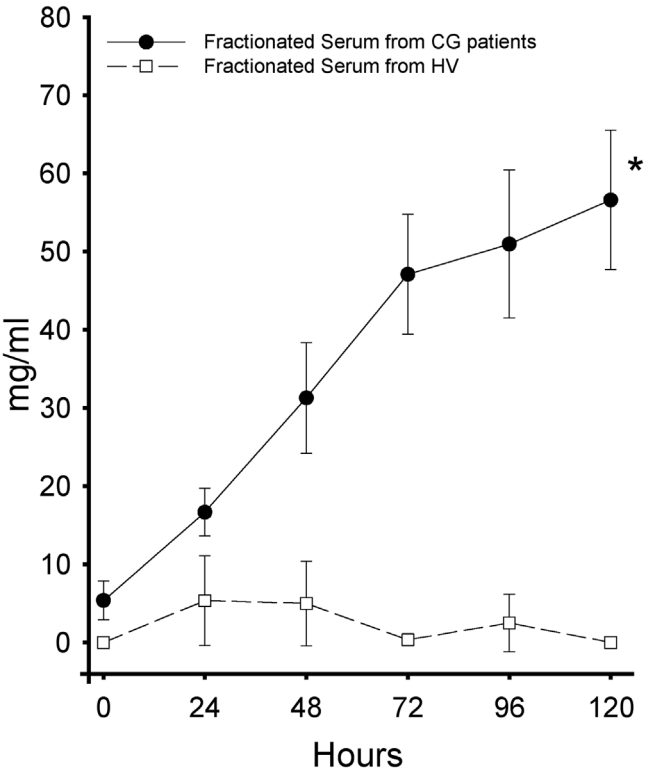
Renal tissue was processed for light microscopy and scanning electron microscopy (Zeiss DSM 950), according to standard techniques (Gonzalez del Pliego et al., 2001). Sections for light microscopy were stained with hematoxilin and eosin (HE), periodic acid-Schiff (PAS), Mallory Trichrome and Jones' methenamine silver stain.

All samples were evaluated for the presence of glomerular collapse, mesangial expansion and/or prominence of the visceral epithelial cells.

Results

Proteinuria

Rats injected with serum from CG patients present an increase in urinary protein excretion 24 hrs after the first injection and at day 5 (120hrs) it reaches a maximum level of 30 mg/ml. Rats injected with serum from healthy subjects remains within the basal level. Urinary creatinine (UCr) and serum creatinine (SCr)



*GC vs HV p<0.001

Fig. 1. Microalbumineuria expressed in mg/ml. Twenty-four hour urinary protein excretion results in rats injected with the fractionated serum from CG patients (Group 1) were seen to be statistically significant (p=0.0001) compared to rats that received serum from healthy subjects (Group 2).

values as well as creatinine clearance in both groups are shown in Table 1 and Fig. 1.

Glomerular histopathology

Rats receiving serum from healthy subjects (Group 2) presented no appreciable damage to the glomerulus by light (Fig. 2A) or scanning electron microscopy (SEM). However rats injected with serum from CG patients presented glomerular damage. Glomerular changes consisted of capillary retraction and mesangial expansion due to the proliferation of mesangial cells. Basement membranes of capillary loops appeared pleated and no inflammatory cells were seen (Fig. 2B).

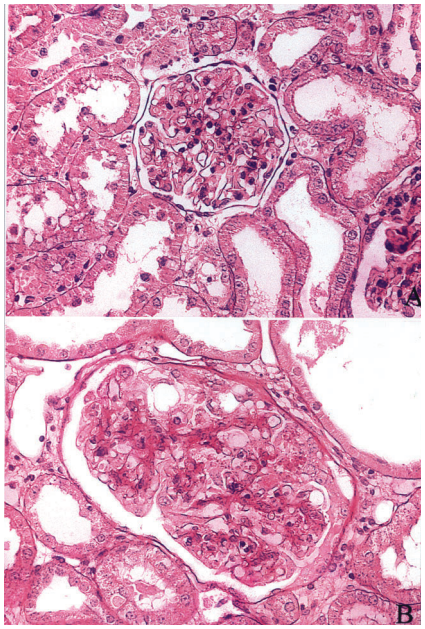


Fig. 2. Glomerulus of rat injected with serum from a healthy subject showing no abnormalities by light microscopy. Capillary loops appear open and basement membranes are fine and delicate. Periodic acid-Schiff stain, x 400. **B.** Glomerulus of rat injected with serum from a CG patient that presents retraction and collapse of the glomerular tuft. Basement membranes of capillary loops appear pleated. Periodic acid-Schiff stain, x 400

Lung histopathology

Patchy mononuclear inflammatory infiltrates were evident in the lung of all rats receiving serum from CG patients (Group 1) Mononuclear infiltrates were integrated exclusively by lymphocytes; (Fig. 3A-C). Patchy lymphocytes surrounding the pulmonary vessel were also observed (Fig. 3C), in some areas, beneath the visceral pleura as seen in figure 3A. In some animals the lymphoid cells affected the bronchioles (Fig. 3D) and infiltrated the respiratory epithelium (Fig. 3E). Lymphoid infiltrates in the wall of bronchioles were appreciated by SEM (Fig. 4). No pulmonary infiltrates or fibrosis by light or SEM were appreciated in rats of group 2.

Discussion

Pulmonary and renal involvement in systemic diseases has been previously reported in diseases such as: Goodpasture's, rheumatic diseases and collagen-vascular diseases, immunodeficiency, bone marrow transplantation and viral infections. Immune etiology is

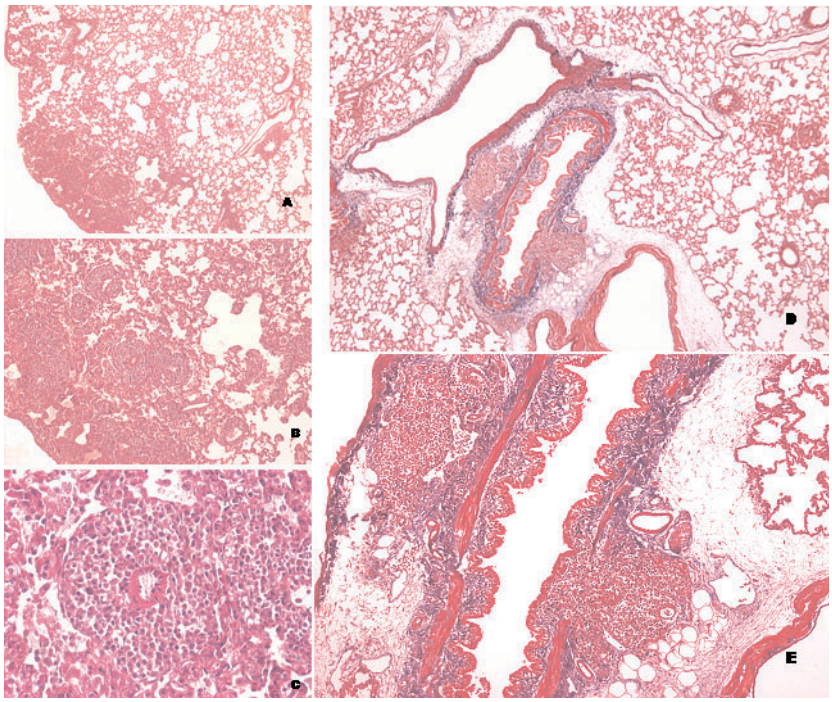


Fig. 3. A. Lymphoid infiltrate beneath visceral pleura is appreciated. Normal parenchyma is also seen PAS, x 40. **B.** Patchy mononuclear infiltrate affecting small vessels is seen. PAS, x 100. **C.** Detailed periarteriolar infiltrate showing exclusively lymphoid cells. PAS, x 400. **D.** Lymphoid infiltrate is also situated around one small bronchiole and small pulmonary vessel. Masson Trichrome stain. x 40. **E.** Infiltrating intraepithelial lymphoid cells are appreciated. Masson Trichrome stain, x 100

usually behind the scene and the clinical pattern observed is an interstitial lung disease with a functional restrictive pattern. Generally, this participation is characterized by lymphoid infiltrates (Fortoul et al., 1985; Travis and Galvin, 2001). Visceral epithelial cell disease including CG, has been associated to T cell disregulation such as parvovirus B-19 infection and systemic lupus erythematosus (Avila-Casado et al., 1998, 2000, 2003; Sharma et al., 2002).

Lymphocytic interstitial pneumonia (LIP) is characterized by diffuse lymphoid infiltration of the alveolar septa. This infiltrate may be considered as an inflammatory and non-neoplastic process and is rarely idiopathic. Other histopathological features are the presence of lymphoid follicles, including those with germinal centers which are located near the bronchioles characteristic of a follicular bronchiolitis entity described in cases of lung participation in connective tissue diseases (Fortoul et al., 1985). The changes found in lungs of this model resemble those reported in the so-called non-neoplastic pulmonary lymphoid lesions as reported in autoimmune diseases (Cushley et al., 1999; Travis and Galvin, 2001). These histological abnormalities might support the hypothesis that those circulating factors affecting the visceral epithelial cell induce the proliferation of lymphoid tissue in the lung.

At this time we have no information about lung participation in this glomerular pathology, maybe, because all the respiratory symptoms are attributable to renal insufficiency and its pathophysiological consequences secondary to fluid overload to the lung (Fortoul et al., 1985; Travis and Galvin, 2001; Wiederkehr et al., 2002).

Collapsing glomerulopathy could present common features with other models of glomerular injury that also share pulmonary participation as we have mentioned previously (Fortoul et al., 1985; Travis and Galvin, 2001; Avila-Casado et al., 2003). More clinical information in human subjects, with special emphasis on lung symptoms, should be taken. In order to improve the patient's life quality, additional treatment could be oriented specifically to the respiratory injury.

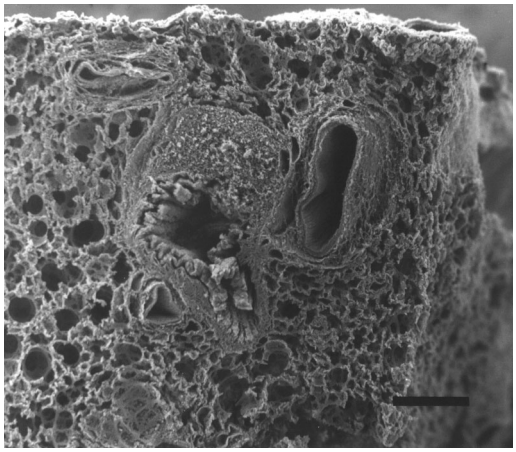


Fig. 4. Patchy lymphoid infiltrate situated between bronchiole and pulmonary artery causing compression of the airway lumen and destruction of the bronchiole wall (SEM). Bar: 149.4 μm.

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