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Impact of lung remodelling on respiratory mechanics in a model of severe allergic inflammation

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Abstract

We developed a model of severe allergic inflammation and investigated the impact of airway and lung parenchyma remodelling on *in vivo* and *in vitro* respiratory mechanics. BALB/c mice were sensitized and challenged with ovalbumin in severe allergic inflammation (SA) group. The control group (C) received saline using the same protocol. Light and electron microscopy showed eosinophil and neutrophil infiltration and fibrosis in airway and lung parenchyma, mucus gland hyperplasia, and airway smooth muscle hypertrophy and hyperplasia in SA group. These morphological changes led to *in vivo* (resistive and viscoelastic pressures, and static elastance) and *in vitro* (tissue elastance and resistance) lung mechanical alterations. Airway responsiveness to methacholine was markedly enhanced in SA as compared with C group. Additionally, IL-4, IL-5, and IL-13 levels in the bronchoalveolar lavage fluid were higher in SA group. In conclusion, this model of severe allergic lung inflammation enabled us to directly assess the role of airway and lung parenchyma inflammation and remodelling on respiratory mechanics.

Keywords: Mammals; Mice; Mechanics of breathing; Elastance; Lung viscoelasticity; Pathology; Lung histology; Eosinophil; Extracellular matrix; Collagen fibres

1. Introduction

The pathology of severe asthma is characterized by a number of structural changes, including epithelial detachment and thickening of the reticular basement membrane (Kay, 1996), mucus gland hyperplasia (Hamid, 2003), subepithelial fibrosis (Hamid, 2003), elastosis and fragmentation of the elastic fibres (Mauad et al., 1999), inflammatory cell infiltration (de Magalhaes Simões et al., 2005), bronchial smooth muscle hypertrophy/hyperplasia (Seow et al., 1998), and vascular changes (Hamid, 2003). Physiologic and pathologic data suggest that these alterations extend beyond the central airways to the distal airways and the lung parenchyma (Carroll et al., 1997; de Magalhaes Simões et al., 2005), making it difficult to obtain tissue samples (Wenzel, 2005). Thus, most of these pathological changes in asthmatic airways have only been investigated postmortem (Kay, 1996).

Murine models of allergic bronchopulmonary inflammation proved to be useful to examine the structural remodelling events, basic mechanisms of allergic inflammation, and the underlying immunologic response (Wagers et al., 2002, 2007; Kumar and Foster, 2002). There are few descriptions of rodent models of severe allergic inflammation (Mukaiyama et al., 2004; Beavitt et al., 2005) and they focused mainly on inflammatory and airway responsiveness processes. Recently, Ochkur et al. developed a double transgenic mouse model with several pulmonary patholo-

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gies representative of severe human asthma. Even though these models described histological and functional data that resembled severe human asthma (Beavitt et al., 2005; Ochkur et al., 2007), none of them studied the consequences of airway and lung parenchyma histological changes on *in vivo* and *in vitro* lung mechanics, not did they correlate the cellular and immune responses linked to the pathologies.

Thus, in the present study we aimed to develop a mouse model of allergic inflammation with histological features of severe human asthma, such as damage of airway epithelium, subepithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, eosinophil and neutrophil infiltration, and mucus gland hyperplasia. We investigated the impact of these pathological changes and cellular and immune modifications on *in vivo* and *in vitro* lung mechanics and methacholine-induced hyperresponsiveness.

2. Materials and methods

2.1. Animal preparation

A total of 62 BALB/c mice (20–25 g) were used. In the severe allergic lung inflammation group (SA) ($n = 8$), mice were immunized, using an adjuvant-free protocol, by the intraperitoneal injection of 10 μg sterile ovalbumin (OVA) in 0.1 ml saline on each of seven alternate days. Forty days after the beginning of sensitization, 40 μg OVA in 20 μl warm sterile saline were intratracheally instilled. This procedure was performed three times with a 3-day interval between them. The control group (C) received saline using the same protocol ($n = 8$). These doses were based on a series of pilot experiments to provide a model of severe allergic lung inflammation with several pulmonary pathologies representative of severe human asthma.

2.2. Measurement of pulmonary mechanics

Twenty-four hours after the last challenge, the animals were sedated (diazepam 1 mg i.p.), anesthetized [pentobarbital sodium (20 mg kg body weight^{-1} i.p.)], and a snugly fitting cannula (0.8 mm i.d.) was introduced into the trachea. Mechanical ventilation (model 683, Harvard Apparatus, Southnatick, MA, USA) with a frequency of 100 breaths min^{-1}, a tidal volume of 0.2 ml, and a positive end-expiratory pressure (PEEP) of 2 cmH_2O was applied. The anterior chest wall was surgically removed.

A pneumotachograph was connected to the tracheal cannula for the measurements of airflow (V') and changes in lung volume (V_T). The pressure gradient across the pneumotachograph was determined by means of a Validyne MP45-2 differential pressure transducer (Engineering Corp, Northridge, CA, USA). The flow resistance of the equipment (Req), tracheal cannula included, was constant up to flow rates of 26 ml s^{-1}, and amounted to 0.12 cmH_2O ml^{-1} s. Equipment resistive pressure ($= \text{Req}V'$) was subtracted from pulmonary resistive pressure so that the results represent intrinsic values. Tracheal pressure (Ptr) was measured with a differential pressure transducer (Engineering Corp, Northridge, CA, USA). All signals were conditioned and amplified in a Beckman type R Dynograph (Schiller Park, IL, USA). Flow and pressure signals were also passed through eight-pole Bessel filters (902LPF, Frequency Devices, Haverhill, MA, USA) with the corner frequency set at 100 Hz, sampled at 200 Hz with a 12-bit analogue-to-digital converter (DT2801A, Data Translation, Marlboro, MA, USA), and stored on a microcomputer. All data were collected using LABDAT software (RHT-InfoData Inc., Montreal, Quebec, Canada).

Muscle relaxation was achieved with vecuronium bromide (0.005 mg kg body weight^{-1} i.v.), and a constant flow ventilator provided artificial ventilation (Samay VR15, Universidad de la Republica, Montevideo, Uruguay). Special care was taken to keep tidal volume ($V_T = 0.2$ ml) and flow ($V' = 1$ ml s^{-1}) constant in all animals in order to avoid the effects of different flows, volumes, and inspiratory duration on the measured variables.

Pulmonary mechanics were measured by the end-inflation occlusion method (Bates et al., 1988). In an open chest preparation, Ptr reflects transpulmonary pressure (PL). Pulmonary resistive ($\Delta P1$), viscoelastic/inhomogeneous ($\Delta P2$) pressures, $\Delta Ptot (= \Delta P1 + \Delta P2)$, and static elastance (Est) were determined. Pulmonary mechanics measurements were performed 10 times in each animal.

Data analysis was performed with ANADAT software (RHT-InfoData Inc., Montreal, Quebec, Canada).

2.3. Measurement of tissue mechanics

Heparine (1000 IU) was intravenously injected immediately after the determination of respiratory mechanics. The trachea was clamped 10 min later at end-expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive haemorrhage that quickly killed the animals. The lungs were removed *en bloc*, and placed in a modified Krebs–Henseleith (K–H) solution (mM: 118.4 NaCl, 4.7 KCl, 1.2 K_3PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2 \cdot H_2O$, 0.6 $MgSO_4 \cdot H_2O$, and 11.1 glucose] at pH 7.40 and 6 °C (Rocco et al., 2001; Xisto et al., 2005). Strips (2 mm × 2 mm × 10 mm) were cut from the periphery of the left lung and suspended vertically in a K–H organ bath maintained at 37 °C, continuously bubbled with a mixture of 95% O_2–5% CO_2 (Lopez-Aguilar and Romero, 1998; Rocco et al., 2001; Xisto et al., 2005). Metal clips made of 0.5 mm-thick music wire were glued to both ends of the tissue strip with cyanoacrylate. One clip was attached to a force transducer (FT03, Grass Instruments Co., Quincy, MA, USA), whereas the other one was fastened to a vertical rod. This fibreglass stick was connected to the cone of a woofer, which was driven by the amplified sinusoidal signal of a waveform generator (3312A Function Generator, Hewlett Packard, Beaverton, OR, USA). A sidearm of the rod was linked to a second force transducer (FT03, Grass Instruments Co., Quincy, MA, USA) by means of a silver spring of known Young's modulus, thus allowing the measurement of displacement. Length and force output signals were conditioned (Gould 5900 Signal Conditioner Frame, Gould Inc., Valley View, OH, USA), fed through eight-pole Bessel filters (902LPF Frequency Devices, Haverhill, MA, USA), analogue-to-digital converted (DT2801A, Data Translation Inc., Marlboro, MA, USA), and stored on a computer. All data were collected using LABDAT