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Mechanical consequences of allergic induced remodeling on mice airway resistance and compressibility

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ABSTRACT

The effect of remodeling on airway function is uncertain. It may affect airway compressibility during forced expirations differently than airflow resistance, providing a tool for its assessment. The aim of the current study was to compare the effects of acute and chronic antigen challenge on methacholine-induced bronchoconstriction assessed from resistance and maximal tidal expiratory flow. Balb/C mice were sensitized with ovalbumin (OVA) and challenged either daily for three days with intra-nasal OVA or daily for 5 days and three times a week for 5 subsequent weeks. Acute and chronic allergen challenge induced airway hyperresponsiveness (AHR) to methacholine. However the relationship between maximal tidal expiratory flow and resistance during methacholine challenge was different between the two conditions, suggesting that the determinants of AHR are not identical following acute and chronic allergen exposure. We conclude that the contrast of changes in maximal tidal expiratory flow and respiratory resistance during methacholine-induced bronchoconstriction may allow the detection of the mechanical consequences of airway remodeling.

1. Introduction

Airway inflammation and smooth muscle are responsible for the clinical features of asthma. With disease progression, remodeling causes airway structural changes that may affect the mechanical properties of the airways and airway responsiveness through geometric effects (Lambert et al., 1993; Wiggs et al., 1990, 1997), changes in tissue biomechanics (Kaczka et al., 1999) and alterations in the interaction between airways and parenchyma (Bates and Lauzon, 2007). Elastic properties of the airways have an important role in determining airflow and are a factor in the explanation of expiratory flow limitation by the tube wave speed theory (Hyatt et al., 1981; Hyatt, 1983). The maximal expiratory flow at a given lung volume is a function of the elastic modulus of the choke point in the airway and extrinsic factors such as lung elastic recoil, the pressure gradient between the alveoli and the choke point as well as upstream airway calibre. Although airway remodeling occurs both in asthma and in COPD, it is not clear exactly how it affects the elastic properties of the airways, due to the difficulty in acquiring in vivo dynamic measurements of the airway properties. Discordance between FEV_1 and flow resistance has been shown in several studies in the context of increased airway wall compliance in bronchiectasis (Hellinckx et al., 1998), but less is known in the context of airway remodeling in asthma. The in vivo measurement of airway wall compliance could help to clarify the role of remodeling in the dynamic behaviour of airways.

Several approaches have been proposed to detecting the changes in airway distensibility, presumably reflecting the effects of remodeling as well as airway smooth muscle tone. The change in anatomic dead space with lung volume has been proposed as a non-invasive index of airway distensibility or stiffness (Fowler, 1948). Using this test the airways of subjects with mild asthma have been shown to be stiffer than normal (Wilson et al., 1993) but the measurement by this technique appears to be dependent on lung volume history and ventilation inhomogeneity (Johns et al., 2006). Recently a forced oscillation technique has been used to evaluate distensibility as the linear slope of conductance versus volume between total lung capacity (TLC) and 75% TLC and between 75% TLC and functional residual capacity (FRC), demonstrating a reduction

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in airway distensibility indices in asthmatic compared to healthy subjects (Browne et al., 2007). These studies measure airway properties during slow inflationary manoeuvres and do not investigate the behaviour of airways during forced expiratory manoeuvres, when airways are compressed and flow limitation occurs. Brackel et al. (2000) showed a decrease in central airway compliance in asthmatic subjects during forced expirations, suggesting a "protective" role of remodeling that increases the mechanical load against smooth muscle contraction.

Studying the behaviour of airways at baseline and following contractile agonist challenge under conditions of tidal expiratory flow limitation may help better understand the role of remodeling in AHR. Contraction of the smooth muscle (ASM) surrounding an airway narrows the airway lumen at a rate determined by the force-velocity behaviour of the muscle and the load against which it has to contract. As the muscle contracts the load against which it contracts increases to a point where it matches the maximum force that muscle is capable of generating and the airway cannot narrow further (Lambert et al., 1993). Changes in the elastic properties of airways and parenchyma likely affect this process determining the magnitude of narrowing, but also the behaviour of the airway when it is acting as a flow-limiting segment. For this reason we developed a method to study airway behaviour under conditions of tidal expiratory flow limitation and to quantify the relation between maximal expiratory flow and resistance in different functional phenotypes of airway pathology using murine models of acute and chronic asthma.

2. Materials and methods

2.1. Animals

Six to eight week old, female Balb/c mice were purchased from Charles River, Canada. Mice were housed in a conventional animal facility under a 12 h light/dark cycle with free access to food and water. A McGill University Institutional Animal Care Committee approved experimental procedures.

2.2. Sensitization and challenge

Mice were sensitized with two intraperitoneal (ip) injections of 10 μg ovalbumin (OVA) in 1 mg aluminium hydroxide at days 0 and 10. Animals were randomly assigned to 4 experimental groups: Acute OVA ($n = 8$), acute PBS ($n = 8$), chronic OVA ($n = 7$), chronic PBS ($n = 8$).

2.3. Acute challenge

Starting at day 17, mice were challenged once a day for 3 days in a custom-built device to constrain and create a nose-only exposure to an ultrasonic nebulisation (Ultra-Neb 100, DeVilbiss Health Care; Somerset, Pa) of 5% OVA in PBS or PBS alone for 30 min. Respiratory mechanics were studied 24 h after the third challenge.

2.4. Chronic challenge

Starting at day 19, mice were challenged once a day for 5 days in a custom-built device to constrain and expose the nose to an ultrasonic nebulisation of 5% OVA in PBS or PBS alone for 30 min and then they received 3 challenges a week for 5 weeks until day 57. Respiratory mechanics were studied 24 h after the last challenge.

2.5. Experimental Setup

On the day of the experiment mice were anaesthetized with xylazine hydrochloride (10 mg/kg, ip) followed by sodium pentobarbital (32 mg/kg, ip). Once anaesthesia was achieved, mice were tracheostomized with an 18 gauge cannula (1.3 cm, typical resistance of 0.2 cm H_2O s/mL; Brico Medical Supplies Inc., Dayton, NJ, USA), connected to a computer controlled small animal ventilator (*flexiVent FX* operated by *flexiWare* v7.0.2 software; SCIREQ Inc., Montreal, QC, Canada) and ventilated with a tidal volume of 10 mL/kg at a frequency 150 breaths/min in a quasi-sinusoidal fashion with a positive end-expiratory pressure (PEEP) of 3 cm H_2O. Before initiating a sequence of baseline measurements, the respiratory system was inflated to a transrespiratory pressure of 30 cm H_2O over a period of 3 s and held at that pressure for the same time period in order to recruit any atelectatic areas of lung and to standardize lung volume history. A muscle relaxant, pancuronium chloride (0.8 mg/kg, ip), was then administered and the animal was allow to stabilise for approximately 2 min.

The mechanical properties of the respiratory system were assessed using single frequency (2.5 Hz; Snapshot-150, 1.2 s) and broadband forced oscillation signals (1–20.5 Hz; Quick Prime-3, 3 s) as well as a quasi-static, step-wise, pressure-controlled pressure-volume loop (PVs-P, 16 s) (McGovern et al., 2013). The linear single-compartment model was used to evaluate total respiratory resistance (R_{rs}) and elastance (E_{rs}) while the constant phase model allowed the partitioning of the responses within the lungs (Hantos et al., 1992). This latter model fits the input impedance as a function of frequency in the equation: $Z_{rs}(f) = R_N + j \times 2\pi f \times I_{aw} + (G - j \times H)/(2\pi f)\alpha$, where Z is input impedance and expresses the combined effects of resistance, compliance, and inertance as a function of frequency (f); R_N is Newtonian "airway" resistance; I_{aw} is airway inertance and is dominated by the mass of gas in the central airways; and impedance of tissue is accounted for by G (tissue damping) and H (tissue elastance). G is closely related to peripheral airway and tissue resistance and reflects energy dissipation in the lung tissues, j is an imaginary number, H is tissue elastance and reflects energy storage in the tissues, α is $2/\pi \tan^{-1}(H/G)$, and f is respiratory frequency (Hantos et al., 1992). The deflation limb of the PV loop was fit to the Salazar–Knowles equation to obtain static compliance (C_{st}), an estimate of the inspiratory capacity (A) and the shape constant k (Salazar and Knowles, 1964).

To assess airway function during tidal expiratory flow limitation a series of ten tidal breaths was generated with each breath having progressively higher imposed expiratory flows while the pattern of inspiratory flow remained unchanged (Figure 1A). The progressive increase in the expiratory flow was created by negative pressure, through a computer-controlled faster retrieval of the ventilator piston. For a 20 g mouse, the inspiratory flow generated was constant at 1.67 mL/s and the expiratory flows were respectively: 1, 2, 3, 5, 7, 8, 10, 12, 15 and 17 mL/s. During the entire duration of the perturbation (2 s), the resulting pressure and volume changes were recorded at the airway opening at a sampling rate of 1024 Hz and the airway opening tidal expiratory flow (F_{aw}) was derived from the gas compression-corrected volume signal (V_{tr}) directly in the system operating software (Figure 1B, C). Peak tidal expiratory flow (Figure 1D) was determined during each of the ten tidal breaths with progressively increasing expiratory flow. A plateau in the peak tidal expiratory flow during the manoeuvre despite an increase in the driving pressure generated by the ventilator piston was considered as expiratory flow limitation and was chosen as the maximal flow.

The impact of imposed progressive negative pressure tidal expirations on respiratory mechanics parameters was assessed under baseline conditions. It was evaluated for the constant phase model parameters (R_N, G, H) by calculating the absolute difference (Δ) in the outcome value obtained from two measurements taken immediately before and after an imposed series of ten tidal breaths with increasingly negative pressure expirations.