

## ORIGINAL PAPER

# Ovalbumin-sensitized mice are good models for airway hyperresponsiveness but not acute physiological responses to allergen inhalation

G. R. Zosky\*, A. N. Larcombe\*, O. J. White\*, J. T. Burchell\*, T. Z. Janosi†, Z. Hantos\*,†, P. G. Holt\*, P. D. Sly\* and D. J. Turner\*

\*Centre for Child Health Research, Telethon Institute for Child Health Research, University of Western Australia, Perth, WA, Australia and †Department of Medical Informatics and Engineering, University of Szeged, Szeged, Hungary

## Clinical and Experimental Allergy

### Summary

**Background** Asthma is a chronic inflammatory disease that is characterized clinically by airway hyperresponsiveness (AHR) to bronchoconstricting agents. The physiological response of the asthmatic lung to inhaled allergen is often characterized by two distinct phases: an early-phase response (EPR) within the first hour following exposure that subsides and a late-phase response (LPR) that is more prolonged and may occur several hours later. Mouse models of asthma have become increasingly popular and should be designed to exhibit an EPR, LPR and AHR.

**Objective** To determine whether a common model of asthma is capable of demonstrating an EPR, LPR and AHR.

**Methods** BALB/c mice were sensitized to ovalbumin (OVA) and challenged with one or three OVA aerosols. Changes in lung mechanics in response to allergen inhalation were assessed using a modification of the low-frequency forced oscillation technique (LFOT). In order to assess AHR, changes in lung mechanics in response to aerosolized methacholine were assessed using LFOT. Inflammatory cell infiltration into the lung was measured via bronchoalveolar lavage (BAL). ELISAs were used to measure inflammatory cytokines in the BAL and levels of IgE in the serum.

**Results** An EPR was only detectable after three OVA aerosols in approximately half of the mice studied. There was no evidence of an LPR despite a clear increase in cellular infiltration 6 h post-allergen challenge. AHR was present after a single OVA aerosol but not after three OVA aerosols.

**Conclusions** The lack of an LPR, limited EPR and the absence of a link between the LPR and AHR highlight the limitations of this mouse model as a complete model of the lung dysfunction associated with asthma.

**Keywords** airway hyperresponsiveness, asthma, early-phase response, late-phase response, mouse model

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### Correspondence:

Graeme R. Zosky, Division of Clinical Sciences, Telethon Institute for Child Health Research, University of Western Australia, 100 Roberts Road, Subiaco, WA 6008, Australia.  
E-mail: graeme.zosky@ichr.uwa.edu.au

### Introduction

Asthma is an allergic disease of the lungs characterized by airway inflammation [1] and increased responsiveness to bronchoconstricting agents [2]. Within minutes of exposure to an inhaled allergen, the asthmatic lung often develops an early-phase response (EPR) that is characterized by acute bronchoconstriction [3]. This process is thought to be driven primarily by the cross-linking of high-affinity surface-bound IgE receptors on mast cells [4]. This leads to mast cell degranulation and the release of inflammatory mediators such as histamine [5]. In approxi-

mately 50% of adult asthmatics, the EPR is followed several hours later by a more prolonged late-phase response (LPR) that is also characterized by airway constriction [3]. This process is less clearly understood but is related to the presence of activated T-helper type 2 cells [6]. Thus, the altered lung physiology of the asthmatic lung can be characterized by a biphasic response to inhaled allergen and airway hyperresponsiveness (AHR) to bronchoconstrictors.

In recent years, mice have become increasingly popular to model human asthma with numerous studies that have examined the altered lung physiology associated with

allergic responses in the airways. In contrast, there have only been a limited number of studies that have examined the physiological response of the lung to inhaled allergen using these models.

Examination of the EPR in mouse models of asthma has implicated factors such as allergen-specific IgG1 [7], nerve growth factor [8] and mast cells [9]. However, these studies have primarily used a non-physiological variable known as enhanced pause (Penh) as their outcome measure of lung function. Penh has been widely criticized as a proxy for airway resistance [10], as it is highly dependent on breathing frequency and susceptible to changes in upper airway flow that are critical factors in mouse lung function as they are obligate nose breathers. This calls into question previous evidence of the presence or otherwise of a physiological EPR in mice and the factors that may be driving this response. Cieslewicz *et al.* [11] used a measure of total lung resistance based on the single compartment equation of motion and demonstrated that it is possible to invoke a physiological EPR in BALB/c mice sensitized to ovalbumin (OVA). However, they used a global measure of Newtonian resistance to characterize lung responses and as such were not able to separate the changes in lung function that they observed into those representing the airway and the lung parenchyma.

Similarly, previous attempts at measuring the LPR have been hampered by the use of Penh as the primary outcome measure [11–16]. These studies have also had mixed results with some studies [11, 13–16] but not others [12] purporting to show a physiological LPR in the lungs of sensitized and challenged mice. This variability may be due to a number of factors such as the problems with the techniques for measuring lung mechanics already mentioned and the lack of assessment of changes in lung volume that are known to influence respiratory mechanics in mice [17]. Again, Cieslewicz *et al.* [11] have provided some data using an appropriate physiological measure of lung function at a single time-point 6 h after allergen challenge but it is unclear whether this constriction was restricted to the airways or whether a single allergen challenge is sufficient to elicit a response.

A majority of studies have focused on AHR to bronchoconstricting agents in mice models of asthma. A number of these models show AHR measured with appropriate techniques [18, 19] and it is clear that AHR can be induced to varying degrees depending on the model that is used [20]. While there are similarities in the type of sensitization and challenge protocol used in these studies and the acute allergen response studies described above, no study has attempted to demonstrate a mouse model of asthma that exhibits AHR using an appropriate measure of lung mechanics that also develops a physiological EPR and/or LPR to allergen challenge.

This study aimed to use a technique that directly assesses changes in lung mechanics to determine whether

one of the most common murine models of asthma has increased responsiveness to bronchoconstricting agents and also develops physiological responses to allergen inhalation.

## Methods

### Animals

Eight-week-old female BALB/c mice obtained from the Animal Resource Centre (Murdoch, WA, Australia) were used for all experiments. Mice were housed in a specific pathogen-free environment with a 12h:12h light dark cycle and provided with an OVA-free diet and acidified water *ad libitum*. All experiments were conducted with the approval of the Institutional Animal Ethics and Experimentation Committee.

### Sensitization

Mice were sensitized on day 0 with an intraperitoneal (i.p.) injection containing 20 µg of chicken egg OVA (Sigma, St Louis, MO, USA) suspended in 200 µL of Alum (Alu-gel-S, Serva, Heidelberg, Germany). All mice received a boost injection with the same dose of OVA and alum on day 14.

### Lung physiology

**Animal preparation.** Mice were anaesthetized with an i.p. injection containing xylazine (2 mg/mL, Troy Laboratories, NSW, Australia) and ketamine (40 mg/mL, Troy Laboratories,) at a dose of 0.01 mL/g. Once a surgical level of anaesthesia was induced (two-thirds of the anaesthetic dose), a tracheostomy was performed and a 10 mm length of polyethylene tubing (1.27 mm OD: 0.86 mm ID; Micro-tube Extrusions, NSW, Australia) was inserted into the trachea and secured with suture. The mouse was then connected to a computer-controlled small animal ventilator (flexiVent, Scireq, Montreal, Canada) and ventilated at 450 b/min with a tidal volume of 8 mL/kg and a positive end-expiratory pressure of 2 cmH<sub>2</sub>O.

**Measurement of respiratory mechanics.** Respiratory mechanics were measured using a modification of the low-frequency-forced-oscillation technique (LFOT) as described previously [21, 22]. For measurement of the EPR, a 16 s oscillatory signal was delivered via the endotracheal tube during pauses in ventilation by the flexiVent piston. The oscillatory signal contained 19 sinusoidal mutually prime frequencies ranging from 0.25 to 19.625 Hz. The respiratory system input impedance spectrum ( $Z_{rs}$ ) was calculated and a four-parameter model [23] was fit to the data points to obtain airway resistance

( $R_{aw}$ ), inertance ( $I_{aw}$ ), tissue damping ( $G$ ) and tissue elastance ( $H$ ). This system allowed for the delivery of allergen directly to the mouse while being ventilated. For measurement of the LPR, an oscillatory signal, containing frequencies ranging from 1 to 25 Hz, was delivered by a loudspeaker in a box set-up via a wavetube [22] and the same four-parameter model applied to the data collected. This system allowed the simultaneous measurement of lung mechanics and lung volume (see below for details).

**Measurement of lung volume.** Thoracic gas volume (TGV) was measured at a trans-respiratory pressure of 0 cmH<sub>2</sub>O as described previously [24]. Briefly, following anaesthesia and tracheostomy, spontaneously breathing mice were placed inside a custom-built plethysmograph and attached to the ventilation circuit (open to the atmosphere until the start of artificial ventilation). At end expiration, the trachea was occluded and spontaneous breathing efforts were recorded for approximately 10 s. TGV was calculated by analysing the tracheal vs. box pressure curves as described previously [24].

### Inflammatory responses

**Bronchoalveolar lavage.** Bronchoalveolar lavage (BAL) fluid was obtained by gently washing a 1 mL solution containing 0.2% w/v bovine serum albumin (BSA) (CSL, Victoria, Australia) to nourish the cells and 0.35% w/v lidocaine (Sigma), to encourage inflammatory cells to slough off the epithelium, in and out of the lungs three times via the tracheal cannula. The samples were centrifuged at 400 g for 4 min and the supernatant was collected for cytokine analysis. The cells were then resuspended in phosphate buffer saline (PBS) and a sample was taken for counting on a haemocytometer to obtain a total cell count. Samples of this resuspended BAL were also spun onto slides and stained with Leishmann's solution for differential cell counts using light microscopy. ELISAs for IL-4, IL-5 and IFN- $\gamma$  were conducted as per the manufacturer's (BD Biosciences, San Diego, CA, USA) instructions.

**Serum antibodies.** Serum samples were analysed for levels of total IgE using an ELISA. Ninety-six-well plates (Nunc, Maxisorp, Denmark) were coated overnight with anti-mouse IgE (BD Pharmingen, San Diego, CA, USA), washed with 0.05% Tween (Sigma) in PBS and blocked with 1% BSA (CSL) in PBS. Standards (purified mouse IgE; BD Pharmingen) and diluted sera samples were then added to the plates and incubated for 1 h at room temperature. Plates were washed six times before adding biotinylated anti-mouse IgE (BD Pharmingen) for 1 h. For analysis of IgG1, plates were coated overnight with OVA (10  $\mu$ g/mL;

Sigma) in carbonate coating buffer at 4 °C and then blocked with 1% BSA in PBS for 30 min. Plates were washed three times with 0.05% Tween in PBS. Standards (mouse anti-OVA; Antibody Shop, Gentofte, Denmark) and diluted sera were added to the plates and incubated for 1 h at room temperature. Plates were washed six times before adding biotinylated rat anti-mouse IgG1 (BD Pharmingen) for 1 h. IgE and IgG1 plates were incubated with Streptavidin-horseradish peroxidase (Amersham, Bucks, UK). An ABTS substrate was added for approximately 10 min before being stopped with 20% SDS (Research Organics, Cleveland, OH, USA). Plates were then read at 405 nm using a Wallac Victor 3 (Wallac Oy, Turku, Finland), and concentrations were calculated by fitting a four-parameter model to the standard curve with Workout software (PerkinElmer, Boston, MA, USA).

### Study protocol

**Methacholine challenge.** On day 21, a group of sensitized mice were placed in a perspex chamber and exposed to a 30-min aerosol of either 1% OVA (w/v in pyrogen-free saline) or pyrogen-free saline delivered by an ultrasonic nebulizer (Ultraneb, DeVilbiss, Somerset, PA, USA). Twenty-four hours later, mice were prepared as outlined above and connected to the ventilator. After 5 min of regular ventilation, three 16-s inflation manoeuvres, separated by 1 min of regular ventilation, from functional residual capacity volume to  $V_{20}$  (lung volume at 20 cmH<sub>2</sub>O) were performed in order to standardize lung volume history. After the lung volume history was standardized, an LFOT measurement was taken every minute for 5 min to establish baseline values for lung mechanics. Five baseline LFOT measures were collected before introducing a saline aerosol into the ventilation circuit for 90 s. An LFOT measurement was collected every minute for 5 min after the saline aerosol before introducing 90-s aerosols of increasing doses (0.1–30 mg/mL) of methacholine (MCh) separated by 5 min of LFOT measurements.

**Early-phase response.** On day 21, a separate group of sensitized anaesthetized, tracheostomized mice were connected to the ventilator. Lung volume history was standardized and baseline LFOT measurements were taken as per the MCh challenge protocol. An aerosol of 5% OVA (w/v in pyrogen-free saline) or saline was then delivered into the ventilation circuit by an ultrasonic nebulizer (Ultraneb, DeVilbiss) for 5 min. LFOT measurements were then taken every minute for 40 min. At the end of the physiology measurements, the mouse was removed from the ventilator and a BAL and serum sample were taken.

**Late-phase response.** Another group of sensitized mice were also placed in a perspex chamber and exposed to a

30-min aerosol of either 1% OVA (w/v in pyrogen-free saline) or pyrogen-free saline delivered by an ultrasonic nebulizer (Ultraneb, DeVilbiss). These animals were then prepared as outlined above and lung function measurements were collected from separate groups of mice 2, 4, 6, 8, 12 or 24 h after the aerosol. TGV was measured as described above, with the mouse making spontaneous breathing efforts against the occluded airway opening. The mouse was connected to the ventilator and, following approximately 2–3 min of ventilation, three static measures of lung function at a trans-respiratory pressure of 0 cmH<sub>2</sub>O, separated by 30 s of regular ventilation, were taken. At the end of each study, a BAL and serum sample were collected.

**Effect of multiple aerosols.** As it was unclear whether a single aerosol would be sufficient to elicit an EPR or LPR in this model and there is some controversy regarding the presence of these acute allergic responses in mice that have been challenged with multiple aerosols, we exposed other groups of sensitized mice to additional aerosols before testing for the presence of an EPR or LPR. These mice were exposed to 1% OVA for 30 min on three consecutive days in a perspex chamber before being tested for an EPR, LPR or MCh responsiveness 24 h after the last aerosol using the protocol outlined above.

### Statistical analyses

All statistical analyses were performed using SigmaStat 3.0 (SPSS Inc). Lung function data on the EPR were compared using repeated measures ANOVA with Holm–Sidak post-hoc tests. All other data were compared using ANOVA with Holm–Sidak post-hoc tests. To satisfy the assumptions of normality and equal variance between groups, the data were transformed where appropriate. Data are reported as mean (SD).

## Results

### Antibodies

IgE and IgG1 were detectable in the sera of all mice in this study. There was no difference in the levels of IgE ( $P=0.609$ ) or IgG1 ( $P=0.870$ ) in the sera of sensitized mice exposed to a single OVA ( $n=7$ ) [IgE 208.3(81.5) ng/mL; IgG1 6353.7(1655.2) ng/mL] or saline ( $n=9$ ) [IgE 235.7(26.1) ng/mL; IgG1 4640.4(1601.1) ng/mL] aerosol in the EPR experiments. Similarly, there was no significant increase in IgE ( $P=0.645$ ) or IgG1 ( $P=0.324$ ) 2, 4, 6, 8, 12 or 24 h after a single OVA aerosol compared with mice exposed to a single saline aerosol (data not shown). Exposing the mice to three additional OVA aerosols ( $n=11$ ) led to an increase in the levels of IgE

[508.9(319.5) ng/mL] ( $P=0.005$ ) and IgG1 [23263.7 (13362.1) ng/mL] ( $P<0.001$ ) in the sera.

### Inflammation and cytokines

Total cell ( $P=0.684$ ) and differential cell counts (macrophages,  $P=0.953$ ; neutrophils,  $P=0.269$ , eosinophils,  $P=0.768$ ) obtained from the BAL did not differ significantly between mice exposed to OVA ( $n=7$ ) [macrophages 53526(20421) cells/mL; neutrophils 908(1179) cells/mL; eosinophils 3116(3630) cells/mL] or saline ( $n=9$ ) [macrophages 58140(9457) cells/mL; neutrophils 2012(2116) cells/mL; eosinophils 1788(2708) cells/mL] on the ventilator in the EPR protocol. There was no significant difference between the number of macrophages ( $P=0.595$ ), neutrophils ( $P=0.237$ ) or eosinophils ( $P=0.474$ ) between timepoints in the saline-challenged mice tested for a LPR after a single aerosol. As such, the saline data were pooled and compared against the OVA groups. Sensitized mice exposed to a single OVA aerosol had a significantly higher total cell count compared with saline-challenged mice that peaked 6 h post-allergen challenge ( $P=0.003$ ) (Fig. 1). The increase in total cell numbers was characterized by a significant ( $P<0.05$ ) increase in the number of neutrophils and eosinophils, which also peaked 6 h post-OVA challenge (Fig. 1). This pattern was mirrored by the Th-2 cascade cytokines IL-4 and IL-5, which were maximal 6 h post-challenge. There was no IFN- $\gamma$  detectable in the BAL of any mice in this study. Mice primed with three OVA aerosols ( $n=15$ ) had significantly higher total cell counts ( $P=0.002$ ), macrophages [119740(80894) cells/mL] ( $P<0.001$ ), neutrophils [53507(34106) cells/mL] ( $P=0.029$ ) and eosinophils [154586(124577) cells/mL] ( $P=0.003$ ) in their BAL than mice primed with one OVA aerosol ( $n=15$ ). There was no significant difference between the levels of IL-5 or IL-4 in mice primed with one or three OVA aerosols (IL-5,  $P=0.887$ ; IL-4,  $P=0.097$ ).

### Lung function

**Methacholine responsiveness.** Sensitized mice exposed to a single OVA aerosol ( $n=8$ ) had significantly higher responses to MCh in  $R_{aw}$  ( $P=0.011$ ) and  $G$  ( $P=0.013$ ) but not  $H$  ( $P=0.166$ ) compared with controls ( $n=7$ ). In contrast, mice primed with three OVA aerosols ( $n=6$ ) had significantly higher responses in  $H$  ( $P=0.021$ ) to MCh, with evidence of an increased response in  $G$  ( $P=0.051$ ) but not in  $R_{aw}$  ( $P=0.385$ ) compared with controls (Fig. 2).

**Early-phase response.** There was no evidence of an EPR following a single OVA challenge given during mechanical ventilation. The values for  $R_{aw}$  remained relatively constant during the 40-min measurement period (Fig. 3), with no significant difference between the OVA ( $n=7$ ) and

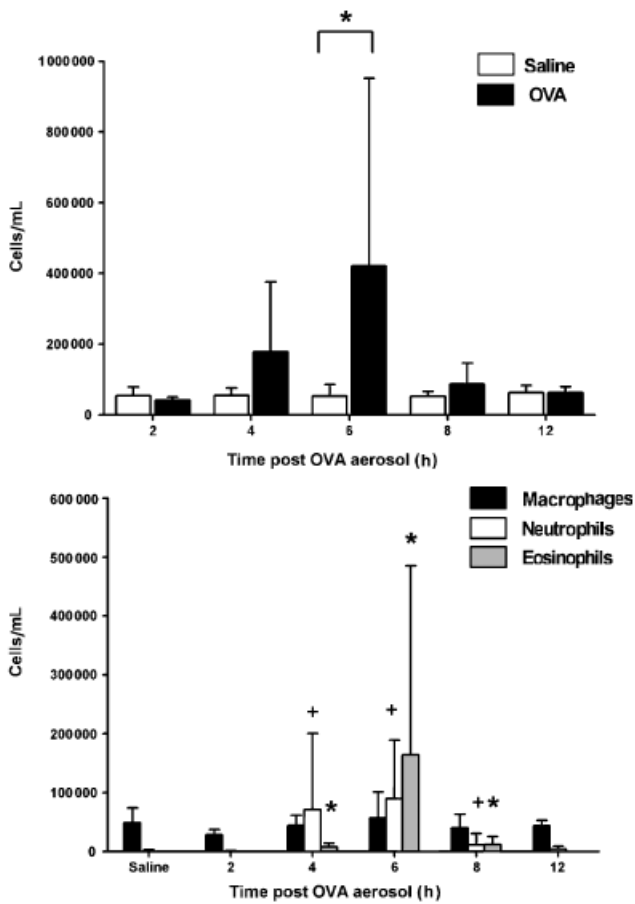


Fig. 1. Total cell counts (top panel) for mice exposed to ovalbumin (OVA) (■) or saline (□) 2, 4, 6, 8 and 12 h post-aerosol. The bottom panel shows differential cell counts from the same mice. Total cell counts were significantly higher ( $P < 0.05$ ) in OVA-exposed mice 6 h post-aerosol compared with saline-mice. The numbers of neutrophils ( $P < 0.05$ ; +) and eosinophils ( $P < 0.05$ ; \*) were significantly higher in OVA mice compared with saline levels 4, 6 and 8 h post-aerosol. Error bars represent the standard deviation.

saline groups ( $n = 9$ ) ( $R_{aw}$ ,  $P = 0.934$ ). There was a slight increase in both  $G$  ( $P < 0.001$ ) and  $H$  ( $P < 0.001$ ) over time; however, there were no significant differences between OVA and saline groups ( $G$ ,  $P = 0.773$ ;  $H$ ,  $P = 0.230$ , respectively) (Fig. 3).

There was evidence in some mice primed with three OVA aerosols of a substantial increase in airway resistance within 10 min of exposure to 5% OVA. On the basis of the variability in the magnitude of the responses in these mice, 'responders' were classified as those that had a percentage increase in  $R_{aw}$  that was 1.96 standard deviations higher than the mean of the maximum response in the saline-challenged group (cut-off percentage of 151.4%). Of the 11 mice challenged with 5% OVA on the ventilator after three priming OVA aerosols, five mice were classified as responders (Fig. 4). There was no significant difference in inflammatory cell counts for mice classified as responders or non-responders ( $P = 0.083$ ).

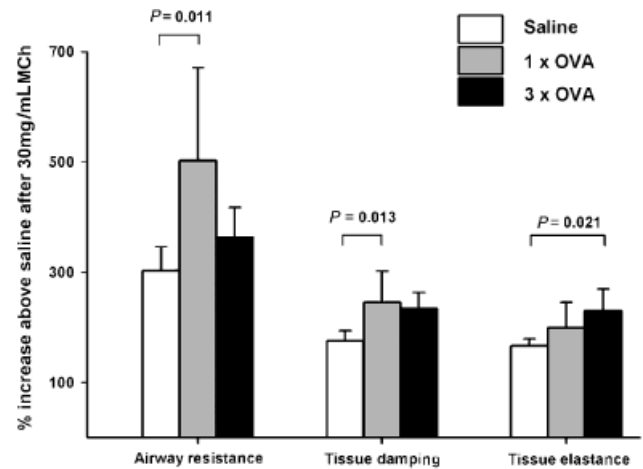


Fig. 2. Mean (SD) increase (expressed as a percentage of the response to saline) in airway resistance, tissue damping and tissue elastance to a 30 mg/mL methacholine (MCh) aerosol in sensitized mice exposed to control ( $n = 8$ ), 1 ovalbumin (OVA) aerosol ( $n = 7$ ) or 3 OVA aerosols ( $n = 6$ ).

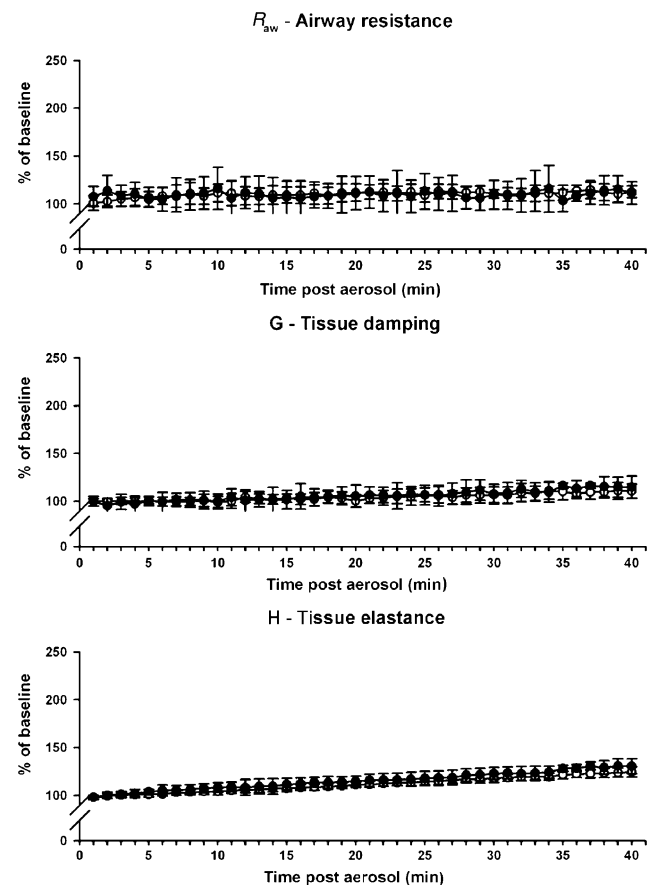


Fig. 3. Tracking of airway resistance ( $R_{aw}$ ), tissue damping ( $G$ ) and tissue elastance ( $H$ ) expressed as a percentage of the baseline measurements following a 5% ovalbumin (OVA) aerosol (●,  $n = 7$ ) or saline aerosol (○,  $n = 9$ ) in mice sensitized to OVA. Data are mean (SD).

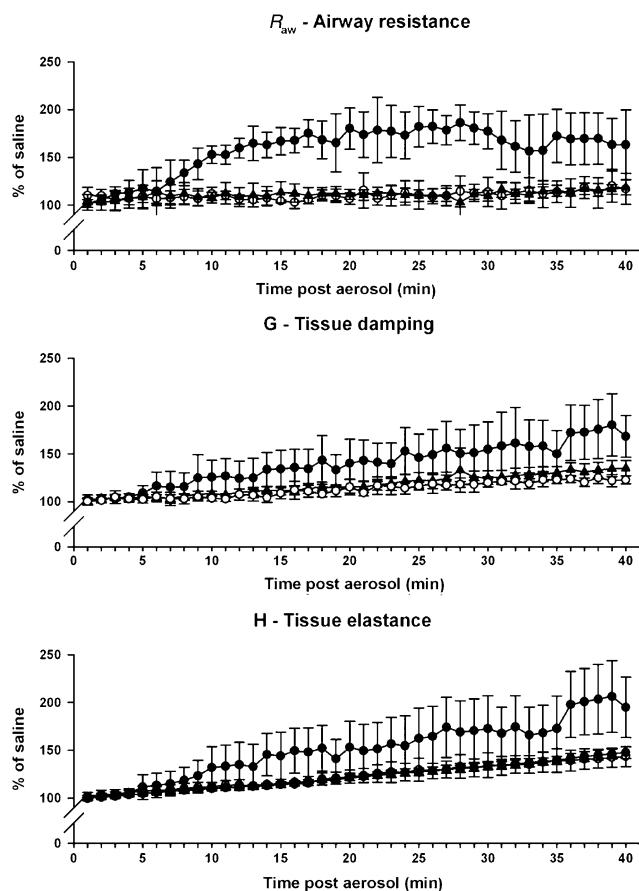


Fig. 4. Tracking of airway resistance ( $R_{aw}$ ), tissue damping ( $G$ ) and tissue elastance ( $H$ ) expressed as a percentage of the baseline measurements in mice primed with three daily 1% ovalbumin (OVA) aerosols for mice exposed to a 5% OVA aerosol that responded ( $\bullet$ ,  $n = 5$ ) or did not respond ( $\circ$ ,  $n = 6$ ), or saline aerosol ( $\blacktriangle$ ,  $n = 4$ ). Data are mean (SD).

Similarly, the levels of IgE ( $P = 0.081$ ) and IgG1 ( $P = 0.439$ ) were not significantly different between mice classified as responders and non-responders.

#### Late-phase response

There was no evidence of an LPR in sensitized mice challenged with a single OVA aerosol. The values for  $R_{aw}$  ( $P = 0.293$ ),  $G$  ( $P = 0.729$ ) and  $H$  ( $P = 0.188$ ) were not significantly different between OVA- and saline-challenged mice at any time-point between 2 and 24 h post-challenge (Fig. 5). There was also no significant difference in TGV measured at 0 transrespiratory pressure between any of these groups of mice ( $P = 0.091$ ) (Fig. 5). The addition of three priming aerosols was not sufficient to induce an LPR in the airways of mice exposed to OVA ( $R_{aw}$ ,  $P = 0.141$ ) compared with mice exposed to saline after three OVA aerosols. And, while there was a significant ( $P = 0.018$ ) but small (OVA: 46982(1205) hPa/L vs. saline: 42839(1174) hPa/L) increase in  $H$  in OVA-exposed mice compared with saline-exposed mice after three priming

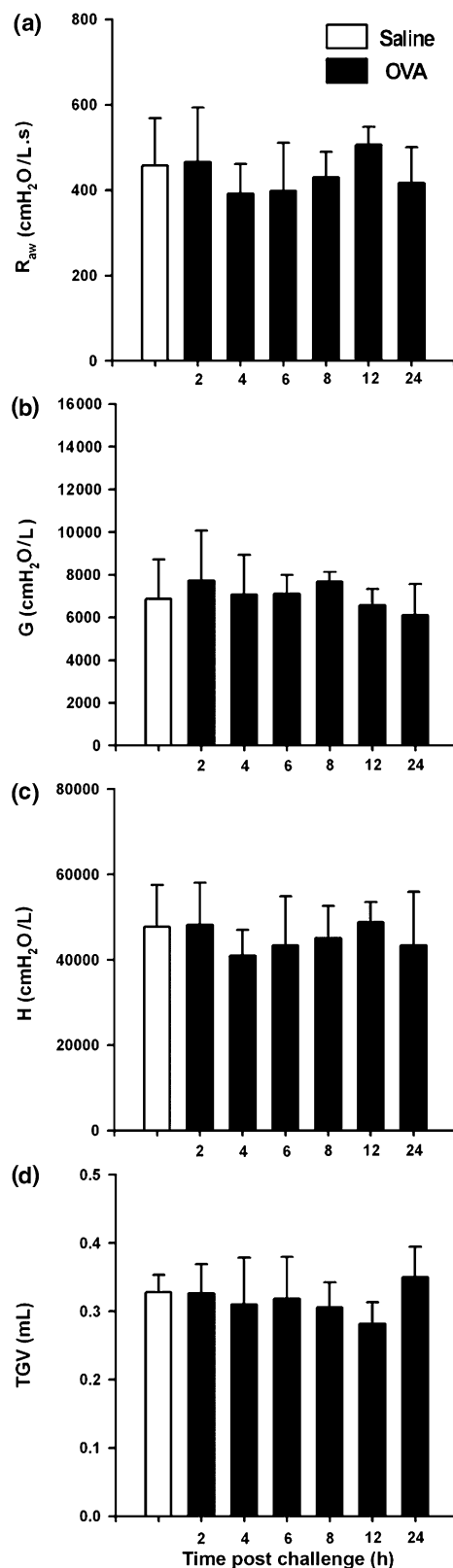


Fig. 5. Mean airway resistance ( $R_{aw}$ , A), tissue damping ( $G$ , B), tissue elastance ( $H$ , C) and thoracic gas volume (TGV) (D) for sensitized mice exposed to ovalbumin (OVA) ( $\blacksquare$ ) or saline ( $\square$ ) 2, 4, 6, 8, 12 and 24 h post aerosol. The error bars represent the SD and there were no significant differences between groups.

aerosols, there was no difference in  $G$  ( $P=0.423$ ) or TGV ( $P=0.991$ ) (data not shown).

## Discussion

The data from the present study are consistent with earlier studies showing that increased responsiveness to MCh can be induced in mice sensitized to a specific allergen after a single allergen challenge [25, 18, 20]. A single OVA aerosol was sufficient to induce an increase in response to MCh but did not result in allergen-induced bronchoconstriction. This was despite clear evidence of a late-phase influx of inflammatory cells into the lung. While it was possible to induce an EPR after multiple OVA aerosols, none of the challenge protocols used resulted in allergen-induced late-phase bronchoconstriction. As a result, it is clear that this particular mouse model of asthma is useful for studying mechanisms relating to increased responsiveness to bronchoconstricting agents but is limited as a model for acute allergen-induced responses.

Previous studies examining both the EPR and LPR in mice have been hampered by the use of inappropriate techniques for detecting changes in lung function following acute allergen challenge. These models, including the one used in the present study, are not only used as models of asthmatic-like reactions in the lung, they are also used as models of allergic rhinitis [26]. Techniques like Penh may be influenced directly by changes in flow in the upper airway and indirectly by changes in breathing pattern that may result from such flow limitation, particularly given that mice are obligate nose breathers. Consequently, any studies that have used Penh as a measure of changes in lung function in response to allergen exposure, irrespective of how well designed they are for examining factors relating to immunological events, may be discounted as it is unclear whether the changes they have observed would in fact relate to the airway constriction associated with the immunological response to allergen challenge.

Cieslewicz et al. [11], in a study aimed at determining the contribution of eosinophils and IL-5 to the EPR and LPR, presented some data using a direct measure of global lung resistance and were able to demonstrate a physiological EPR and evidence of an LPR 6 h post-challenge. The timing of these responses correlated with their data using Penh; however, the remainder of these data that were used to interpret the contribution of IL-5 and eosinophils were presented as relative changes in Penh. As such, for the reasons outlined above it is difficult to determine whether the conclusions that they reached about the changes in inflammatory responses reflected actual changes in the constriction of the airways.

The need for three priming challenges in the present study to elicit an EPR is consistent with Cieslewicz et al. [11]. Our data provide clear evidence that it is at least

possible to induce a physiological EPR in BALB/c mice sensitized to OVA, which is reflected by changes in the conducting airways ( $R_{aw}$ ) and the parenchyma ( $G$ ,  $H$ ). The mechanism of the need to prime the inflammatory response with allergen challenges before it is possible to induce an EPR in this model is unclear and requires further investigation. Similarly, an explanation for the variability in the response, with only five of the 11 mice studied responding to acute allergen challenge, is unclear, with no evidence of differences in levels of sensitization (IgE, IgG1) or inflammation and cytokine activity. This kind of variability in response to allergen exposure in sensitized animals has been reported previously in other animal models [27]. These authors were able to demonstrate the role of endogenous corticosterone in modifying the response to allergen in rats and it is possible that a similar mechanism is involved in our study.

In contrast to the EPR, we were not able to demonstrate any evidence of a physiological LPR. While we were able to generate an EPR in mice with a prior level of inflammation in the airway, this was not the case with the LPR. The pattern of inflammation in terms of an influx of eosinophils and increased levels of Th-2 cascade cytokines with a peak approximately 6 h post-allergen exposure is consistent with previous reports in other mouse models [11], although others have found maximum inflammation 12–24 h post-allergen exposure [16]. Such discrepancies in timing are prevalent in the literature and in this case may be explained by the difference in the type of allergen used between the two studies. This lack of a physiological LPR is consistent with other studies that were not able to demonstrate a physiological LPR despite prior exposure to priming allergen challenges [12]. Although this similarity, for the reasons outlined previously, should be treated with caution due to the differences in techniques for measuring lung function. This limited evidence for an EPR and complete lack of evidence for a LPR may not be surprising, given previous studies showing that mice readily bronchoconstrict to muscarinic agonists but not other mediators such as histamine and leukotrienes [28], which may be critical for the development of acute changes in lung physiology in response to allergen exposure. At the very least, the present study has clearly demonstrated a dissociation between acute inflammatory events, as indicated by increased inflammatory cells in the BAL, and bronchoconstriction in this model of asthma.

One issue that needs to be acknowledged with respect to the design of these experiments is the discrepancy between the concentration of OVA used in order to test for an EPR while on the ventilator and the concentration of OVA used for the priming and LPR experiments. A 1% OVA aerosol delivered over a 30-min period was chosen because it represents one of the most common methods for inducing an allergic airways response in systemically sensitized mice and has been used by us previously to

demonstrate AHR [20]. As the EPR may occur within minutes of allergen exposure, the mice needed to be mechanically ventilated to allow measurement of changes in lung mechanics while the allergen was delivered to the lung. Under these circumstances, it would have been inappropriate to deliver the antigen over a 30-min period as it is not possible to make measurements of lung mechanics while an aerosol is being delivered to the ventilation circuit. In addition, under these circumstances, the allergen would be delivered more efficiently to the airway compared with the aerosol delivered to the conscious spontaneously breathing mice, meaning that a shorter aerosol was required to deliver the same quantity of OVA to the lung. In preliminary experiments, a 1% OVA aerosol for 2 min was used but due to a lack of response this was increased to 5%, which was sufficient to illicit a response in mice primed with three OVA aerosols. Concentrations of OVA above this level were not possible due to the viscosity of the preparation. Despite the differences in concentration used for the EPR experiments, the results from this study have clearly shown the limitations of mice systemically sensitized with OVA as models for acute bronchoconstriction in response to allergen exposure.

The decision to examine the LPR with a single time-point in anaesthetized, tracheostomized mice was based on a number of factors. Firstly, there are very few techniques that are able to measure, satisfactorily, lung mechanics that partition changes in respiratory impedance into airway and parenchymal compartments [29]. One of the downsides of more direct and accurate techniques is the need for anaesthesia, surgical manipulation and having to measure lung mechanics with the animal in the supine position. Such interventions may have an influence on the physiological appropriateness of the preparation but can provide the most detailed information about lung mechanics. This problem led Bates and Irvin [30] to describe the 'phenotyping uncertainty principle,' whereby the most accurate measurement techniques require the least 'physiological' preparation. Rather than sacrifice accuracy, we chose a more invasive technique that was able to determine detailed information about lung impedance and lung volume. In order to avoid the problem of long-term anaesthesia and ventilation, which can lead to significant atelectasis and lung injury after only a few hours in mice [31], we chose to measure physiology at regular intervals in different groups of mice. This method provided the most accurate examination of lung physiology and at the same time minimized the problems associated with mechanical ventilation and anaesthesia.

Our rationale for monitoring TGV during the measurement of the LPR was the possibility that changes in lung volume may create a false impression of allergen-induced changes in lung mechanics. In the present study, it was clear that TGV did not change in mice challenged with

OVA compared with saline at any of the time-points measured. Consequently, it was clear that the lack of evidence of an LPR in the lung mechanics parameters was not due to any change in lung volume as a result of an acute allergic response, despite evidence of a substantial inflammatory response in the BAL after a single OVA exposure. The consistency of our results, with relatively small standard deviations, supports the validity of this technique and also serves to highlight the complete lack of evidence of a physiological LPR in this mouse model of asthma.

The LPR in humans is thought to be linked to the increased responsiveness to bronchoconstricting agents that is characteristic of asthma [32, 33]. The present study has shown a distinct dissociation between AHR and the physiological response to allergen inhalation in this model of asthma. A single challenge was sufficient to induce a significant increase in responsiveness in the airways to inhaled MCh 24 h after challenge. On the basis of the results of the present study, there are at least two major discrepancies between this particular mouse model and the human condition. Firstly, there is a complete lack of evidence of a physiological LPR. Secondly, there is no correlation between the response to allergen and the development of increased responsiveness to MCh. That is, after a single aerosol there is no evidence of constriction due to the allergen itself (EPR or LPR) and yet this is sufficient to induce a significant increase in responsiveness to MCh.

There have been numerous reviews discussing the merits or otherwise of mice as models for this disease [34–38]. A number of these reviews have focused on the differences in immunology between mouse models of asthma and humans, which has been an area of vigorous debate at times [39, 40]. One factor that should be kept in mind in such a debate is the importance of the deficits in lung function, which are characterized by substantial flow limitation in the airways, and their influence on the mortality and morbidity associated with asthma. From this point of view, the model of asthma used in the present study appears to be lacking, with only a limited physiological response to allergen inhalation that is usually the most overt, and is certainly the most clinically relevant, response in human asthmatics. However, the mouse as a model in general does have significant advantages in terms of its use in mechanistic studies in understanding disease progression [41]. This ability to tease out individual mechanisms is one of the primary reasons for their popularity in this field. Consequently, these differences between mouse models and human asthmatics could be seen as an advantage in terms of our understanding of the dissociation between physiological responses to allergen and the development of increased responsiveness to bronchoconstricting agents. One thing is clear: there are limits to any model system and extrapolating data from

these models requires an in-depth understanding of both their limitations and their advantages.

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