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Temporal profile of forced expiratory lung function in allergen-challenged Brown–Norway rats

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Abstract

The Brown–Norway rat is often used to study the allergic pulmonary response. However, relatively little is known about the delayed phase reactions after allergen challenge in this species. To evaluate the temporal changes in lung function and elucidate the mechanisms involved in the delayed phase response, Brown–Norway rats were sensitized and challenged to aerosolized ovalbumin and lung functions were measured by forced expiratory maneuvers and forced oscillation for up to 10 days after a single antigen challenge. Statistically significant ($P<0.05$) reductions in inspiratory capacity, forced vital capacity, functional residual capacity, peak expiratory flow and maximum mid-expiratory flow and increases in respiratory system resistance and elastance were seen by 1 to 3 days after ovalbumin challenge that returned to baseline by 10 days. The reductions in lung function after ovalbumin challenge were blocked by the corticosteroid, betamethasone (1 mg/kg, p.o.). Histological evaluation of lung tissue of sensitized rats demonstrated evidence of interstitial pulmonary edema, an increase in tissue eosinophils and an increase in Periodic Acid Schiff-positive cells in the airway epithelium. Bronchoalveolar lavage fluid samples showed large numbers of eosinophils and increased mucin content up to 6 days after antigen challenge. There was also an increase in wet-to-dry lung weight ratio in the lungs of sensitized rats after antigen. These results demonstrate that prolonged reductions in lung function occur after a single antigen challenge in Brown–Norway rats that is probably due to inflammatory processes producing interstitial pulmonary edema, mucus secretion and cellular influx into the lungs.

Keywords: Allergic inflammation; Forced expiratory maneuver; Forced oscillation; Edema; FRC (Functional Residual Capacity); FVC (Forced Vital Capacity); IC (Inspiratory Capacity); MMEF (Mean Mid-Expiratory Flow); Cchord; Lung parenchyma; Goblet cell; (Brown–Norway rat)

1. Introduction

The pathology of human asthma, a disease predominantly the result of a Th-2 driven allergic inflammation, is characterized by various changes in the airways including mucus plugging, shedding of epithelial cells, thickening of the basement membrane, engorgement of the pulmonary vessels, angiogenesis, airway edema, eosinophilic inflammatory cell infiltration, and smooth muscle hypertrophy and hyperplasia (Hogg, 1993). Additionally, human asthmatics demonstrate abnormal pulmonary functions including reversible airflow obstruction, airway hyperresponsiveness and obstruction in peripheral airways (Ahmed et al., 1981; Kraft, 1999). Several animal models of human asthma have been developed in an attempt to induce the pathology and pathophysiology seen in the human conditions (Elwood et al., 1991; Kung et al., 1994). One of the most widely used models is the Brown–Norway rat model of airway allergy because many of the pathological features of human asthma are seen in this model after a single (Schneider et al., 1997) or multiple (Xu et al., 2002) antigen challenges. These features include the presence of eosinophilic lung inflammation, damage to the airway epithelium, thickening of the basement membrane, acute and late phase airflow obstruction and the presence of airway hyperresponsiveness after the antigen challenge (Eidelman et al., 1988; Elwood et al., 1991; Schneider et al., 1997; Xu et al., 2002).

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Although the Brown–Norway rat is widely used for studies of the allergic pulmonary response, limited information exists on the mechanisms and duration of the late phase allergic response in this species. Eidelman et al. (1988) used the measurement of airway resistance to show the presence of airflow obstruction up to 12 h after antigen challenge. Lin et al. (2000) used a forced expiratory maneuver technique to show a reduction in mid-expiratory airflow by 1 day after antigen challenge. However, a detailed analysis of lung function beyond 1 day after antigen challenge has not been performed in the Brown–Norway rat. Furthermore, no studies have attempted to correlate changes in the lung pathology with altered lung functions during the late phase response in this species.

In the present study, pulmonary functions were measured in allergen challenged Brown–Norway rats using the techniques of forced expiratory maneuvers (Lin et al., 2000, 2002) and forced oscillation (Pétak et al., 1999; Hall et al., 1999) for up to 10 days after antigen challenge. Studies were also performed after treatment with the corticosteroid, betamethasone to evaluate the effect of this anti-inflammatory drug on the pulmonary changes induced by the antigen challenge. Furthermore, a detailed analysis of lung histology, cellular content of the broncho-alveolar lavage and wet-to-dry weight ratio of the lungs was conducted at different times after the antigen challenge to investigate the mechanisms responsible for the antigen-induced alterations in lung function.

2. Materials and methods

2.1. Animals

Inbred male Brown–Norway rats weighing 150 to 200 g were obtained from Charles River laboratory (Wilmington, MA). The rats were housed three per polycarbonate cage; each cage was supplied with hardwood chip bedding and a filter top and placed in an animal colony room maintained at 20–22 °C. The animals were allowed food and water ad libitum. The study was conducted with prior approval from Animal Care and Use Committee of Schering-Plough Research Institute, a facility accredited by the American Association for the Accreditation of Laboratory Animal Care.

2.2. Sensitization and antigen bronchoprovocation

Animals were sensitized by an intra-peritoneal (i.p.) injection of 1 ml alum-precipitated antigen containing 20 μg of ovalbumin (grade III; Sigma Chemical Co., St Louis, MO) and 8 mg of aluminum hydroxide gel (alum) suspended in 0.9% NaCl solution. A booster injection of this alum–ovalbumin mixture was given 7 days later. Non-sensitized animals were injected with aluminum hydroxide gel only. Seven days after the second injection, animals were exposed to aerosolized antigen bronchoprovocation which was performed by placing the rats into a closed plexiglass chamber (21 l) and exposing the rats to aerosolized ovalbumin (1%) for 15 min. The aerosolized ovalbumin was produced by an ultrasonic nebulizer (DeVilbiss, Somerset, PA, USA; Model Ultra-Neb 99) at a flow rate of approximately 8 l/min.

2.3. Measurement of lung function

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and surgically prepared with a tracheal cannula. A commercially available forced expiratory maneuvers system was used to measure lung volumes and airflows (Buxco Electronics, Troy, NY, USA). The rats were placed inside a whole body plethysmograph and the tracheal cannula was connected to an outlet port. A computer driven breathing valve was connected to the tracheal outlet port that allowed for the rats to breathe spontaneously or was used for forced inflations and deflations of the lungs. Lung inflations were produced using air from a compressed air source (21% O_2, balance N_2) and deflations were produced using a vacuum line. A wire mesh screen was placed into a 1-in. diameter opening in the top of the plethysmograph and airflow across this screen was made using a differential pressure transducer (±2 cm H_2O, Validyne Corp., California, USA). Volume was measured by electronic integration of the airflow signal and a volume calibration was performed before each experiment using a 3-ml syringe. Airway opening pressure was measured directly from the breathing valve using a differential pressure transducer (±70 cm H_2O, Motorola Inc., California, USA).

The rats were placed inside the plethysmograph and after 5 min of spontaneous respiration, four forced deep breaths were performed to provide a standardized volume history. This was produced by inflating the lungs until the airway opening pressure increased to 10 cm H_2O. When the animal resumed spontaneous respiration, the tracheal port was occluded for 15 s at end expiration whereupon the animal made spontaneous respiratory efforts against the occluded airway. Functional residual capacity (FRC) was measured using Boyle's Law equation from the corresponding changes in pressure and thoracic gas volume (Levitzy, 1999). The rats were then entrained to artificial ventilation (tidal volume = 10 ml/kg and respiratory frequency = 60 breaths/min) and 5 min later, four forced deep breaths were administered to standardize the volume history. The lungs were then inflated until the airway opening pressure reached 20 cm H_2O. This was followed by rapid (about 0.2 sec) deflation of the lungs, performed by switching the breathing valve to the vacuum source which was set at a pressure of −40 cm H_2O. The end of the deflation procedure was produced when expiratory flow value decreased to 0.5 ml/s. This maneuver was performed three times in each rat and the results expressed in each animal as the average of these three determinations.

The above-mentioned procedures allowed the measurement of inspiratory capacity (IC), forced vital capacity (FVC), expiratory reserve volume (ERV), peak expiratory flow (PEF), and mean mid-expiratory flow (MMEF). Subsequently, the total lung capacity (TLC) was calculated from the values of IC and FRC. Likewise, residual volume (RV) was derived by subtracting ERV values from FRC. Additionally, three forced expiratory maneuvers were performed in each rat inflating the

lungs to an airway opening pressure of 20 cm H_2O followed by a relatively slow deflation over a 4 sec period. From this procedure, pulmonary "chord" compliance (Cchord) was measured between 0 and 10 cm H_2O.

In a separate cohort of animals, respiratory system resistance (Rrs) and elastance (Ers) were measured using the technique of forced oscillation. The anesthetized rats were prepared with a tracheal catheter and artificially ventilated (tidal volume = 10 ml/kg, respiratory frequency = 60 breaths/min) with a computer assisted ventilator (Flexivent ®, Scireq Inc., Montreal, QC, Canada). The ventilator was used to deliver two cycles of a 2 Hz volume sinusoidal waveform to the airway. Airway pressure and delivered volume were measured continuously and airflow was calculated from the derivative of volume. A two element series resistance–elastance model for the lung was fitted to the flow, volume and pressure data to yield values for Rrs and Ers.

2.4. Experimental procedures

Three experiments were performed to assess the changes in lung function over time following antigen challenge. In the first experiment, aerosolized ovalbumin challenge was performed in non-sensistized and sensitized rats and forced expiratory lung functions of FVC and MMEF were measured at 10 min, 6 hr and 24 hr after the ovalbumin challenge ($n = 8$ per group). In the second experiment, forced expiratory lung functions of FVC, IC, TLC, PEF, MMEF and Cchord were measured at 1, 3 and 10 days after the ovalbumin challenge. The third experiment was performed using the forced oscillation technique and Rrs and Ers were measured at 1, 3 and 10 days after the ovalbumin challenge.

Experiments were performed in rats treated with the corticosteroid, betamethasone to assess whether the changes in lung function induced by the antigen challenge were attenuated by treatment with this anti-inflammatory drug. In these studies, sensitized rats were orally dosed with betamethasone (1 mg/kg) given once a day for 2 days prior to the aerosolized ovalbumin challenge followed by two more doses on the third day at 1 h before and 5 h after the ovalbumin challenge. Forced expiratory lung functions were evaluated 24 h after the ovalbumin challenge. Responses in steroid-treated rats were compared to rats orally dosed with 0.4% methylcellulose ($n = 8$ per group). Historical data from our laboratory have found this dosing regime to completely inhibit the inflammatory cell influx into the lungs of allergen-challenged Brown–Norway rats.

To correlate the temporal changes in lung function with inflammatory conditions in the lungs, bronchoalveolar lavage fluid and lung histological sections were obtained from both sensitized and non-sensitized rats at different times after antigen challenge. The rats were sacrificed at the end of each study and a tracheal catheter was inserted. Bronchoalveolar lavage was collected by lavaging the lungs with 2 aliquots of 5 ml of 0.9% NaCl solution. Total recovery volume per rat was approximately 8 ml. The total cell count in the bronchoalveolar lavage was performed using a Coulter Cell Counter® (Coulter Z1, Beckman Coulter Inc., Miami, FL). For differential white cell count, cytospins were prepared with 200 µl of bronchoalveolar lavage fluid using a cytocentrifuge (Shandon Inc., Pittsburgh, PA, USA) at 250 revolutions per minute for 10 min. The air-dried cytospins were stained with Leukostat stain (Fisher Scientific, Pittsburgh, PA, USA). Cells were identified as eosinophils, neutrophils, mononuclear and epithelial cells by standard morphology and 200 cells counted under ×400 magnification. The remaining bronchoalveolar lavage fluid was centrifuged at 1600 revolutions per min for 10 min at 4 °C. The supernatant was stored at −80 °C for estimation of mucin content.

Mucin content of the bronchoalveolar lavage fluid was quantified using an enzyme-linked immunosorbent assay using methods similar to that previously described (Phillips et al., 2006). One hundred microliter of bronchoalveolar lavage fluid sample diluted 1:5 with Tris buffered saline was plated in duplicate in microplate wells (Nalge Nunc International, Denmark). Pooled monkey bronchoalveolar lavage fluid (defined as 100 plate units/ml) was used as a positive control. The plate was blocked with 1% bovine serum albumin/

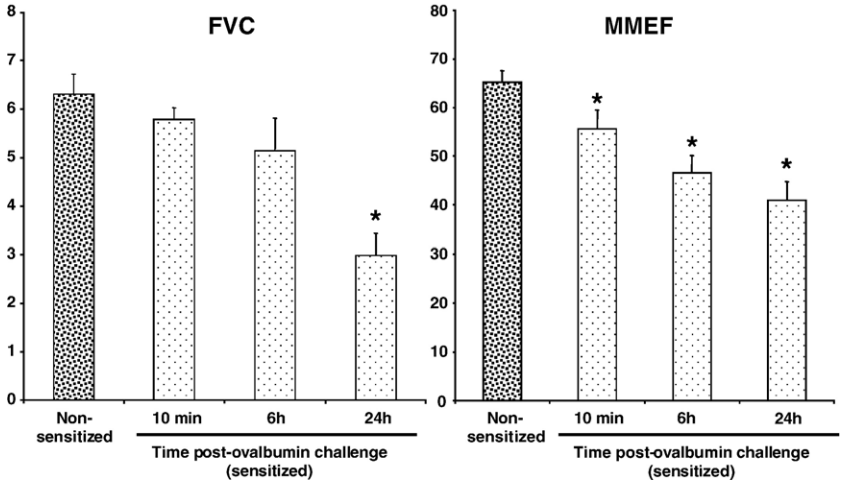

Fig. 1. Temporal change in FVC and MMEF in non-sensitized (▨) and sensitized (□) rats up to 1 day after aerosolized ovalbumin challenge. *Significantly ($P \leq 0.05$) different from non-sensitized animals.

Table 1
Temporal change in forced expiratory lung functions over 10 days after aeroisolized ovalbumin challenge to sensitized and non-sensitized rats

Parameter	1 day post challenge		3 day post challenge		10 day post challenge	
	Non-sensitized	Sensitized	Non-sensitized	Sensitized	Non-sensitized	Sensitized
Capacities and volumes						
IC (ml)	4.2±0.4	2.2±0.1 *	4.4±0.3	3.5±0.3 *	3.9±0.2	4.1±0.3
FVC (ml)	5.0±0.5	2.7±0.2 *	5.2±0.4	3.9±0.4 *	4.6±0.2	5.1±0.5
TLC (ml)	6.9±0.5	4.5±0.2 *	7.8±0.6	6.3±0.4 *	6.6±0.2	7.1±0.5
Expiratory airflows						
PEF (ml/s)	89.7±4.7	70.2±2.3 *	90.1±6.2	73.1±10.5	83.0±7.1	91.3±3.2
MMEF (ml/s)	63.6±5.0	49.3±3.1 *	66.5±3.2	45.0±7.3 *	55.5±2.9	63.4±2.6
Lung mechanics						
Cchord (ml/cm H_2O)	0.3±0.02	0.2±0.01 *	0.3±0.02	0.3±0.02	0.3±0.01	0.3±0.02

Values are means±S.E.M. obtained from 8 non-sensitized and 8 sensitized animals at each time point. IC: inspiratory capacity; FVC: forced vital capacity; TLC: total lung capacity; PEF: peak expiratory flow; MMEF: mean mid expiratory flow; Cchord: compliance between 0 and 10 cm H_2O.
* Significantly ($P \leq 0.05$) different from corresponding non-sensitized group.

phosphate buffered saline for 30 min. This was followed by sequential incubation with primary antibody A10G5 (mouse anti-mucin, 1:300 diluted with 1% bovine serum albumin/phosphate buffered saline) and a goat anti-mouse IgG antibody (diluted 1:5000 with 1% bovine serum albumin/phosphate buffered saline). A reporter system based on an enzyme complex amplification using Streptavidin/horseradish peroxidase conjugate (diluted 1:5000 with 1% bovine serum albumin/phosphate buffered saline) was used to enhance the sensitivity of the assay. The wells were then incubated with chromogen tetramethylbenzidine followed by treatment with sulphuric acid to stop the reaction. Absorbance values were read on a Microplate Spectrophotometer (Modular Devices, Sunnyvale, CA) at 450 nm and 540 nm.

For the histological evaluation of lungs, the rats were prepared with a tracheal catheter and the lungs and trachea were dissected free of surrounding tissue. The lungs were then fixed by instillation of 10% buffered formalin through the tracheal cannula at 20 cm H_2O pressure to ensure proper inflation. The tissues were processed routinely for paraffin embedding. The sections were cut at 5-μm thickness and were stained with hematoxylin and eosin. The sections were also stained with Periodic Acid Schiff (PAS) stain to identify and quantify mucus-producing cells. The hematoxylin and eosin-stained sections were utilized for quantification of eosinophils. The eosinophils were quantified in the peribronchiolar areas of five bronchioles selected randomly on the lung section from each animal, using image analysis system (Northern Eclipse version 5.0, Empix Imaging System, Mississagua, ON, Canada). After digitizing the image, the system was threshold for eosin color. The area around the airway was traced manually and the eosinophils lying within the traced peri-bronchial space were enumerated. Values are represented by an average value from determinations on 5 different airways. Quantification of Periodic Acid Schiff-positive cells was done by calculating the percentage of these cells out of total epithelial cell counted manually.

To assess the wet-to-dry weight ratio of the lungs after antigen challenge, the rats were sacrificed and the lungs were dissected free from surrounding tissues and weighed. The wet-to-dry lung weight ratio was calculated as previously described (Stephens et al., 1988).

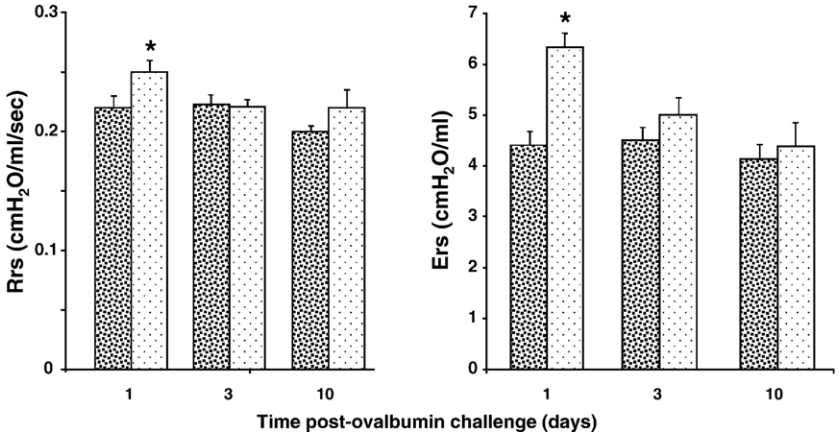

Fig. 2. Temporal change in Rrs and Ers in non-sensitized (▨) and sensitized (□) rats up to 10 days after aerosolized ovalbumin challenge. *Significantly ($P \leq 0.05$) different from non-sensitized animals.

Table 2
Effect of betamethasone on the ovalbumin-induced reduction in forced expiratory lung functions in sensitized rats

Parameter	Non-sensitized	Sensitized	Sensitized + betamethasone
Capacities and volumes			
IC (ml)	4.0±0.4 *	2.6±0.3	4.0±0.2 *
FVC (ml)	4.7±0.5 *	2.8±0.4	4.9±0.3 *
TLC (ml)	7.4±0.5 *	4.8±0.4	6.5±0.2 *
Expiratory airflows			
PEF (ml/s)	86.3±6.7 *	63.5±3.9	96.4±5.5 *
MMEF (ml/s)	54.3±6.2 *	39.4±3.9	69.9±3.2 *
Lung mechanics			
Cchord (ml/cm H_2O)	0.3±0.03 *	0.2±0.02	0.3±0.02 *

Values are means±S.E.M. obtained from non-sensitized and sensitized animals (n=8 per group) 1 day after ovalbumin challenge. Rats orally dosed with betamethasone (1.0 mg/kg) or 0.4% methylcellulose vehicle (see Materials and methods for dosing paradigm).
* Significantly ($P \leq 0.05$) different from sensitized group.

2.5. Statistical analysis

All data are presented as arithmetic means and standard error means. An analysis of variance test with Fisher's protected least significant difference post hoc test was used to analyze the data for statistical significance using Statview version 5.0.1. The results were considered significant if the p value was less than or equal to 0.05.

3. Results

3.1. Temporal profile of antigen-induced alterations in lung function

Aerosolized ovalbumin challenge to sensitized rats produced a small reduction in FVC (8%) and MMEF (15%) measured 10 min after the challenge (Fig. 1). There were further reductions in these lung functions over time and by 24 h after ovalbumin challenge, the reduction in FVC averaged 46% and the reduction in MMEF averaged 22% compared to the values obtained in non-sensitized control rats (Table 1). Statistically significant ($P<0.05$) reductions in IC, TLC, PEF and Cchord were also seen by 1 day after ovalbumin challenge in sensitized rats (Table 1). The reductions in these lung functions persisted for up to 3 days after the ovalbumin challenge but by 10 days after challenge, they had returned close to the baseline values (Table 1). A significant reduction in Cchord was also seen 1 day after ovalbumin challenge to sensitized rats, which returned to baseline by 3 days after challenge (Table 1).

Aerosolized ovalbumin challenge to sensitized rats produced significant increases in Rrs (14%) and Ers (48%) by 1 day after the challenge (Fig. 2). By 3 and 10 days after ovalbumin challenge, there were no differences between non-sensitized and sensitized rats for both Rrs and Ers.

3.2. Effect of betamethasone on ovalbumin-induced alterations in lung function

Treatment of sensitized rats with betamethasone (1 mg/kg, p.o.) blocked the reductions in FVC, IC, TLC, PEF, MMEF and Cchord seen 1 day after ovalbumin challenge (Table 2).

3.3. Temporal profile of bronchoalveolar lavage cell count and mucin content

There was a significant increase in the total cell and eosinophil counts in the bronchoalveolar lavage of sensitized rats compared to values in ovalbumin challenged non-sensitized controls (Fig. 3). This increase started as early as 6 h after the ovalbumin challenge, peaked between 2 and 3 days after ovalbumin challenge and was still significantly higher by day 6 after ovalbumin challenge. However, 10 days after ovalbumin challenge, the number of bronchoalveolar lavage eosinophils in sensitized rats was not different from the non-sensitized control group. A significant increase was also seen in the number of bronchoalveolar neutrophils for up to 3 days after the ovalbumin challenge. However, by 6 and 10 days after the ovalbumin challenge, the number of neutrophils in the

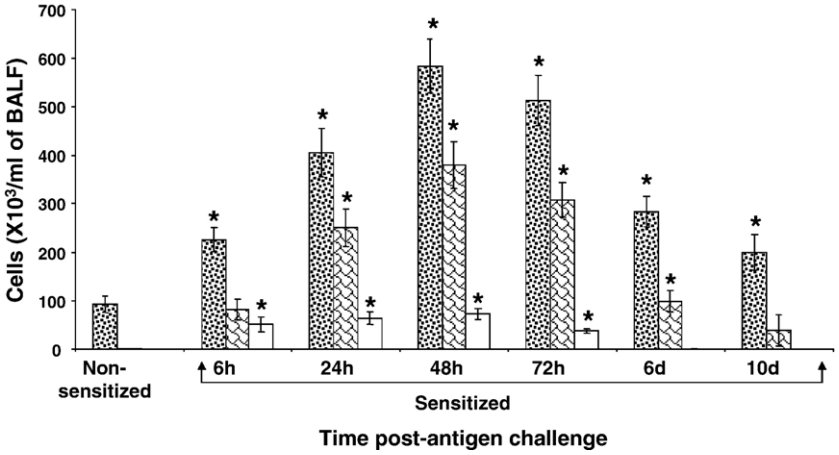

Fig. 3. Temporal change in total cells (▨), eosinophils (▩), and neutrophils (□) in the bronchoalveolar lavage of non-sensitized and sensitized rats up to 10 days after aerosolized ovalbumin challenge. *Significantly ($P \leq 0.05$) different from non-sensitized animals.

Table 3
Temporal profile of ovalbumin-induced alterations in goblet cell population of airway epithelium and mucin contents of bronchoalveolar lavage fluid

Treatment groups	PAS-positive cells (%)	Mucin (plate unit)
Non-sensitized+1 day post ovalbumin	21±3	99±13
Sensitized+1 day post ovalbumin	36±3 *	319±37 *
Sensitized+2 days post ovalbumin	46±2 *	281±43 *
Sensitized+3 days post ovalbumin	41±3 *	259±44 *
Sensitized+6 days post ovalbumin	46±2 *	403±42 *

* Significantly ($P \leq 0.05$) different from non-sensitized group.

bronchoalveolar lavage was identical to that of non-sensitized controls (Fig. 3).

There was a significant increase in the mucin content of the bronchoalveolar lavage collected 1 day after ovalbumin challenge in sensitized rats. This increase in mucin content persisted for 6 days after ovalbumin challenge and correlated with the increase in Periodic Acid Schiff-positive cells in the bronchial epithelium of sensitized rats after the ovalbumin challenge (Table 3).

3.4. Temporal profile lung histological changes

Eosinophils appeared in the peribronchiolar regions of the lungs of sensitized rats beginning as early as 6 h after challenge and persisted for up to 6 days after challenge (Fig. 4). By 10 days after challenge, eosinophilic infiltration around the airways was not different from that measured in the lungs of non-sensitized controls.

There was a marked engorgement of the interstitial space around the large blood vessels with edematous fluid starting as early as 6 h after ovalbumin challenge to sensitized rats. This response persisted for up to 1 day after the challenge. However, no accumulation of fluid was seen in the peri-bronchial area at this time. By 1 day after ovalbumin challenge, large edematous patches were found in the lungs of sensitized rats, particularly around the peribronchial region (Fig. 4B). By 3 days after challenge, increased perivascular and peribronchial spaces along with eosinophilic infiltration in peribronchial and alveolar spaces were still present (Fig. 4C); however, by 10 days, the histological appearance of the perivascular and peribronchial space of sensitized rats was close to that from non-sensitized-control animals (Fig. 4D).

3.5. Wet-to-dry lung weight ratio

The wet-to-dry lung weight ratio 1 day after ovalbumin challenge in sensitized rats (4.9±0.1) was significantly ($p=0.0002$) increased above that seen in nonsensitized rats challenged with ovalbumin (4.5±0.1). In these experiments, the wet lung weight in the non-sensitized group averaged 1.20±0.14 g and in the sensitized group was 1.62±0.15 g. This amounted to a 35±2% increase in wet lung weight after the ovalbumin challenge in sensitized rats.

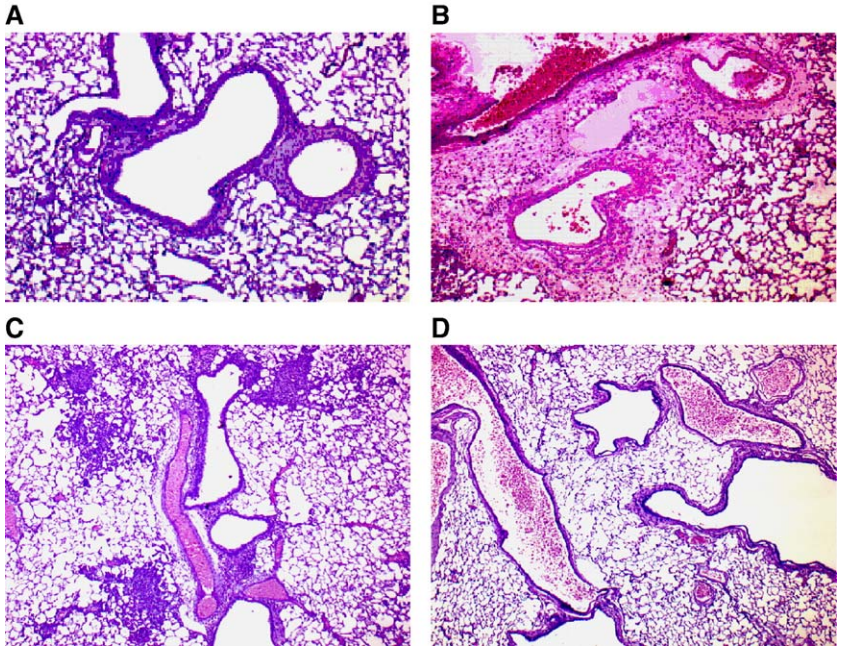

Fig. 4. Light micrographs of the lungs sections (stained with hematoxylin and eosin) from non-sensitized (A) and sensitized animals at 1 day (B), 3 days (C) and 10 days (D) after aerosolized ovalbumin challenge. (A) Lung section from a non-sensitized animal showing well inflated alveoli and airways with no fluid accumulation around the blood vessel and airways, magnification ×28. (B) Lung section from a sensitized animal 1 day after ovalbumin challenge showing significant accumulation of protein-rich fluid both around the blood vessels and airways, magnification ×28. (C) Lung section from a sensitized animal 3 days after antigen challenge showing increased perivascular space, infiltration and accumulation of eosinophils around the airways as well as in the alveoli, magnification ×18. (D) Lung section from a sensitized animal 10 days after ovalbumin challenge showing no histological evidence of fluid accumulation as well as no eosinophilic infiltration, magnification ×18.

4. Discussion

Although Brown–Norway rats are widely used for studies of allergic pulmonary response, relatively little is known about the late phase reactions in this species both in terms of the temporal changes in lung function after antigen challenge and the mechanisms producing this response. The goal of this study was to characterize the late phase pulmonary response to antigen challenge in the allergic Brown–Norway rat and to further investigate mechanisms responsible for these altered pulmonary responses. Using techniques of forced expiratory maneuvers and forced oscillation to evaluate the pulmonary response to the ovalbumin challenge, significant reductions in IC, FVC, TLC, MMEF and PEF and increases in Rrs and Ers were found after a single ovalbumin challenge to sensitized rats with the largest reductions in lung function occurring 1 day after the ovalbumin challenge. The reductions in lung function were completely blocked by pretreatment with a corticosteroid demonstrating that this response is produced by the actions of inflammatory mediators. An evaluation of the lung histology and cellular content of the bronchoalveolar lavage fluid demonstrates the presence of inflammatory cell infiltration, interstitial airway edema and an increased mucin content. The presence of interstitial pulmonary edema was confirmed by an increase in the wet-to-dry lung weight ratio.

A variety of methods have been used to measure lung functions in the rat (Diamond and O'Donnell, 1977; Watanabe et al., 1995; Pétak et al., 1999; Lin et al., 2000, 2002; Hele et al., 2001; Underwood et al., 2002) and in this study, we used techniques of forced oscillation (Pétak et al., 1999; Hall et al., 1999) and forced expiratory maneuvers (Lin et al., 2000, 2002). The latter method involves inflation of the lungs to TLC and evacuation of the lungs to RV using a vacuum source. Different laboratories have used different inflation pressures such as 20 cm H_2O (Diamond and O'Donnell, 1977), 30 cm H_2O (Sanchez-Cifuentes et al., 1998) and 35 cm H_2O (Lin et al., 2000), to inflate the lungs to TLC. In our pilot studies, inflation pressures of 20, 30 and 40 cm H_2O were used to determine the optimum pressure to be used for these experiments. In these experiments, the largest magnitude change in lung function after antigen challenge was produced using the 20 cm H_2O inflation pressure. Although inflation pressures of 30 and 40 cm H_2O were found to inflate the lungs maximally and yield larger values for TLC, the relative change after antigen challenge was found to be less at the higher lung inflation pressures. Deep inspiration is known to reduce airway and lung tissue impedance (Fish et al., 1981) and it is possible that the effects of antigen are partially reversed with higher degrees of lung inflation.

Aerosolized ovalbumin challenge to sensitized rats produced a progressive decline in lung functions that showed the largest change by 1 day after challenge. Using the technique of forced expiratory maneuvers, relatively large reductions were seen in FVC and IC with somewhat smaller reductions occurring in the parameters of expiratory airflows, such as PEF and MMEF. These results were corroborated by measuring Rrs and Ers via the forced oscillation technique which show relatively large increases in lung elastance (Ers) with much smaller increases in airflow resistance (Rrs). By 3 days after the ovalbumin challenge, these lung capacities and expiratory airflows were still reduced below baseline values but Rrs and Ers had returned to baseline values. By 10 days after ovalbumin challenge, all lung functions had returned to normal. Using the forced expiratory maneuvers technique, Lin et al. (2000) also found a reduction in expiratory airflow 1 day after ovalbumin challenge in Brown–Norway rats. Our results demonstrate that the reductions in expiratory flows and lung volumes persist for at least 3 days after the ovalbumin challenge and return to baseline by 10 days.

The mechanisms responsible for the impairment of lung function after the ovalbumin challenge were also investigated in the study. The fact that the reductions in lung function occurred days rather than hours after the antigen challenge suggest that this response is not mediated by the actions of acute phase allergic mediators but is due to the actions of a prolonged inflammatory response. The inflammatory nature of this reaction was confirmed in this study using the corticosteroid, betamethasome that completely blocked the reductions in lung function occurring 1 day after antigen challenge. Histological evaluation of the lungs of sensitized rats demonstrated massive accumulation of edematous fluid in the perivascular and peribronchial lung regions 1 day after ovalbumin challenge. The presence of the edema persisted for at least 3 days after ovalbumin challenge. This overall temporal profile of interstitial lung edema coincides with the impairment in lung function and previously published results using magnetic resonance imaging technology to visualize lung edema in the antigen-challenged Brown–Norway rat (Tigani et al., 2003). Confirmation of the presence of lung edema after antigen challenge was seen in our study using the method of an increased wet-to-dry lung weight ratio (Stephens et al., 1988; Pulido et al., 1999; Schneider et al., 1997; Kaner et al., 2000).

The presence of interstitial lung edema would likely cause an increased lung elastance, thereby requiring a larger inflation pressure to expand the lungs to a given volume. Conversely, inflating the lungs to a fixed inflation pressure, i.e. 20 cm H_2O, will result in lower lung volumes when interstitial lung edema is present. Furthermore, if the lungs are inflated at reduced lung volumes, expiratory airflows will be reduced. In other words, the presence of interstitial edema would impact significantly on lung volumes expiratory airflows and lung elastance which is exactly the lung function profile observed in this study.

Another factor that may have contributed to the changes in lung function could be an increase in the number of Periodic Acid Schiff staining mucus-producing goblet cells in the airway epithelium and secreted mucin in airway lumen. An increase in the number of goblet cells and mucin protein following allergen challenge has been previously reported following antigen challenge in Brown–Norway rats (Tesfaigzi et al., 2000). Increased perivascular and peribronchial cuffing with inflammatory cells and edematous fluid, concomitant with metaplasia of goblet cells, can lead to partial airway obstruction. Furthermore, presence of mucus plugs in the airway lumen can directly reduce the luminal diameter. Taken together, these

factors can collectively contribute to the reductions in lung volumes, capacities and obstruction to airflow, as observed in the present study.

In summary, allergen challenge to sensitized Brown–Norway rats produces changes in lung function parameters, which persist for several days after the ovalbumin challenge. The mechanism of the antigen-induced decline in lung function likely involves the development of interstitial pulmonary edema and possibly mucus hyper-secretion. Furthermore, the reduction in the lung function following antigen challenge appears to be solely a result of antigen-induced inflammation since the changes could be completely blocked by pre-treating the animals with betamethasone.

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