



Airway responsiveness in an allergic rabbit model

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ABSTRACT

Background: Animal models of allergy and bronchial hyperresponsiveness (BHR) are useful in researching pulmonary diseases and evaluating drug effects on the airways. Neonatally immunised rabbits exhibit several features of asthma as adults, including early and late airway responses following antigen challenge, oedema and inflammatory cell infiltration into the lung, BHR to inhaled histamine and methacholine (compared with naïve rabbits) and exacerbations of BHR following antigen challenge. Therefore this model can be used to investigate the underlying mechanisms of BHR and for the preclinical evaluation of new drugs for the treatment of asthma. **Aim:** To describe the characteristics of airway responses in a rabbit model of allergic inflammation and to evaluate the relationship between skin test reactivity to antigen, airway inflammation and BHR. **Methods:** New Zealand White rabbits were neonatally immunised against *Alternaria tenuis*. At 3 months of age, airway responsiveness was measured to aerosolised histamine, methacholine or allergen. Bronchoalveolar lavage (BAL) was performed and cell counts recorded. Direct skin tests were performed to assess skin reactivity to allergen and passive cutaneous anaphylaxis (PCA) tests were performed to determine titres of circulating IgE. **Results:** In a population of allergic rabbits, allergen challenge induced a significant bronchoconstriction, airway inflammation and BHR. Skin test responsiveness to allergen did not correlate with various indices of pulmonary mechanics e.g. baseline sensitivity to methacholine and histamine, or allergen-induced BHR. In contrast, skin test responsiveness did predict airway inflammation as assessed by measurements of eosinophil recruitment to the lung. **Conclusion:** The allergic rabbit is a useful model to further our understanding of allergic diseases. Recording lung function using a minimally invasive procedure allows repeated measurements to be made in rabbits longitudinally, and each animal may thus be used as its own control. Our observations do not support the use of skin responsiveness to allergen as a predictor of airway sensitivity as we observed no correlation between skin sensitivity and airway responsiveness to inhaled histamine, methacholine or allergen. However, skin reactivity did predict airway inflammation as assessed by measurements of eosinophil recruitment to the lung. Our results also further highlight the likely dissociation between airway inflammation and BHR.

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1. Introduction

Asthma is a chronic disease characterised by a variety of features, including increased airways responsiveness, airway inflammation and reversible airways obstruction. Various animal models have been established to try and better understand specific aspects of the human disease. Whilst no single animal model is able to reproduce all the features of human asthma, animal models remain useful.

Guinea pigs have traditionally been the most commonly used small animal species for studying airway diseases, as receptor pharmacology in the guinea pig closely matches that of human receptors compared with other small species and many of the existing drugs currently used

in the treatment of airway diseases have been extensively studied in guinea pigs (Bos et al., 2007; Harrison et al., 2008; Keir, Spina, & Page, 2002). However, disadvantages of the guinea pig model are a lack of transgenic models and a limited number of guinea pig strains for comparative work. Mice are increasingly being used to study genetic factors involved in disease pathophysiology and are also being used in the investigation of airway remodelling and inflammation (Pitchford et al., 2004). Genetic manipulation and short breeding times are an advantage of the mouse, but there are some key differences in lung structure and function compared with human airways which limits their suitability to the study of human diseases, particularly airways diseases (Persson, 2002).

Rabbits immunised within 24 h of birth preferentially produce antigen specific immunoglobulin E (IgE) antibodies (Pinckard et al., 1977). Animals sensitised in this way exhibit several features of the asthma phenotype. They undergo both early and late airway responses following antigen challenge (Coyle et al., 1989; Murphy et al., 1986; Shampain, Behrens, Larsen, & Henson, 1982). This is associated with

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oedema and inflammatory cell infiltration (Larsen, Wilson, Clark, & Behrens, 1987; Marsh, Irvin, Murphy, Behrens, & Larsen, 1985). Allergic rabbits also exhibit bronchial hyperresponsiveness (BHR) to inhaled histamine and methacholine compared with naïve rabbits (Bloom, Baumgartner-Folkerts, & Palmer, 1988) and also show exacerbations of airways responsiveness following challenge with antigen or inflammatory mediators such as platelet activating factor (PAF) (Coyle et al., 1989; Larsen et al., 1987; Marsh et al., 1985). Repeated measurements of lung function are readily made in the anaesthetised rabbit, and each animal may therefore be used as its own control (Minshall et al., 1993). We have studied the rabbit over many years and in doing so have evaluated the advantages and limitations of this model. We have collated and analysed our large data set to provide a detailed assessment and analysis of characteristics of this model. In addition we have used this model to investigate the relationship between skin test sensitivity to antigen and numerous other variables such as the magnitude of the early asthmatic response (EAR), late asthmatic response (LAR), extent of any airway eosinophilia and whether allergic skin reactivity can predict airway responses.

A major component of the asthma phenotype is atopy. Skin sensitivity to allergen as measured by allergen titration in the skin is often used to select subjects with asthma for entry into clinical trials to assess the clinical effectiveness of novel agents for the treatment of asthma (DuBuske, 2006; Finnerty, Holgate, & Rihoux, 1990), to select asthmatic subjects for genotyping studies (Knudsen, Thomsen, Nolte, & Backer, 2009) and to assess asthma severity (Harmanci, Bakirtas, & Turkas, 2008).

Several studies have pointed to a possible relationship between the degree of allergen sensitivity in the skin and airway hyperresponsiveness to the spasmogens histamine or methacholine and inhaled allergen (Bryant, Burns, & Lazarus, 1975; Cockcroft et al., 1979). BHR to both histamine (Cockcroft, Murdock, Kirby, & Hargreave, 1987) and methacholine (Fowler & Lipworth, 2003) has been associated with the presence of atopy and an understanding of the relationship between BHR and atopy has been identified as fundamental to further our understanding of asthma (Woolcock & Peat, 2000). Furthermore, clinically it has been reported that there is an excellent correlation between the PC₂₀ for histamine and methacholine, and consequently these agents have been used interchangeably, and have been suggested to be useful markers for disease severity or for assessing the effects of drugs on BHR (Cockcroft et al., 2005).

It has also been reported that both the severity of the early asthmatic response to allergen and airway responsiveness to histamine or methacholine can be predicted from the skin-sensitivity to that allergen, as can airway responsiveness to histamine (Cockcroft et al., 1987) or methacholine (Cockcroft et al., 2005). Furthermore, a relationship between skin test sensitivity to allergen and allergen-induced airway inflammation assessed by sputum eosinophilia has also been suggested (Boulay, Lemieux, Deschesnes, & Boulet, 2003).

We have collected a considerable body of data on airway responsiveness to inhaled histamine and methacholine and airways responses to inhaled allergen in allergic rabbits. Here, we report how the airway responsiveness and allergen-induced airway inflammation relate to skin reactivity to allergen and serum IgE titres as markers of allergic status, to determine whether the variability of airway responsiveness between individuals is related to allergic status.

2. Materials and methods

2.1. Animals

New Zealand white (NZW) rabbits (2–3 kg) of either sex were obtained from Froxfield farms UK (Petersfield, Hampshire, UK) or Highgate Farm (Lincolnshire, UK). All procedures described in this paper were subject to Home Office approval and were performed under the Animal (Scientific Procedures) Act, 1986.

2.2. Immunisation protocol

Rabbits were immunised with an extract of *Alternaria tenuis* in aluminium (Al(OH)₃) gel (Minshall et al., 1993), and which was first established as a model of allergic pulmonary inflammation in this species (Shampain et al., 1982). *A. tenuis* extract (0.5 ml of a solution containing 40,000 protein nitrogen units (PNU)/ml) was mixed with sterile Al(OH)₃ moist gel (0.5 ml) and saline (1 ml). Each rabbit received 0.5 ml of this allergen–adjuvant mixture within 24 h of birth by the intraperitoneal route. The injection was repeated weekly for the first month of life and then bi-weekly until 13 weeks of age. Sham immunised littermates received an i.p. injection of 0.5 ml Al(OH)₃ and saline in the ratio of 1:3. A third group of animals received an i.p. injection of 0.5 ml saline only (saline group). This was repeated as described above. Allergic rabbits used in this study were 3 months of age when they were transferred from the breeding unit to our laboratory.

2.3. Measurement of pulmonary function

Measurements of pulmonary function and BHR were made in spontaneously breathing rabbits under neuroleptanalgesia induced by one of 2 methods. Either the animals were sedated with diazepam (2.5 mg/kg, i.p.) and then anaesthetised with an intramuscular (i.m.) injection of Hypnorm™ (0.4 ml/kg), anaesthesia was then maintained throughout the course of the experiment by further administration of Hypnorm™ every 20 min. Alternatively, they were administered ketamine hydrochloride (0.35 mg/kg) and xylazine (0.25 mg/kg) intramuscularly (i.m.) and anaesthesia was maintained with an i.m. injection of a 3:2 mixture of ketamine and xylazine every 30 min. The rabbits were then placed supine and were intubated with a 3.0 mm internal diameter cuffed endotracheal tube which was attached to a heated (37.5 °C) Fleish pneumotachograph (size 00) connected to a Validyne differential pressure transducer (model MP 45-14-87; Validyne Engineering Corp; CA, USA) from which measurements of flow and tidal volume were obtained. A latex oesophageal balloon attached to a polyethylene cannula (internal diameter 1.67 mm; outer diameter 2.42 mm) was inserted into the lower third of the oesophagus and attached to a second differential pressure transducer, connected between the balloon and atmospheric air, from which values of transpulmonary pressure (TPP), the difference between atmospheric and pleural pressure, were derived. The position of the balloon was adjusted in order to maximise the TPP recorded and it then remained in this position throughout the experiment. The flow was integrated to give continuous recording of tidal volume. Values for total lung resistance (R_L; cmH₂O/L/s) and dynamic lung compliance (C_{dyn}; ml/cmH₂O) were calculated using an on-line respiratory analyser (Pulmonary Monitoring System (PMS) Version 5.1; Mumed Ltd, London, UK) on a breath by breath basis.

2.4. Measurement of airway responsiveness to spasmogens

Airway responsiveness was measured in response to inhaled methacholine hydrochloride or histamine diphosphate. The spasmogens were aerosolised in an ultrasonic nebuliser (deVilbiss Ultraneb 99; DeVilbiss Healthcare Ltd, Heston, UK) and delivered directly into the lungs via the endotracheal tube. Initially, baseline lung function parameters were measured, followed by the administration of aerosolised 0.9% physiological saline for 2 min. Following the saline administration, further lung function parameters were recorded and these served as the baseline measurement with which to compare responses to inhaled spasmogens, which were also administered for a 2-min period at each concentration. Doubling concentrations of the spasmogens were cumulatively administered. Histamine diphosphate was administered at doses of 1.25–80 mg/ml and methacholine hydrochloride 0.31–20 mg/ml. The provocation concentration (PC) that produced a 50% increase

in total lung resistance (R_L , PC_{50}) and that producing a 35% decrease in dynamic lung compliance (C_{dyn} , PC_{35}) were determined for each rabbit by linear interpolation and these values were used as indices of airway responsiveness.

2.4.1. Allergen challenge

Rabbits were challenged (by inhalation) with an aerosol of *Alternaria tenuis* extract (20 000 PNU/ml) in 0.9% saline by use of a DeVilbiss nebuliser. The antigen challenge consisted of a 2 min aerosol of saline, followed by two 2-min antigen aerosols, then 4 consecutive antigen aerosols of 4 min duration (total 20 min). After each challenge, respiratory function was recorded as described above. This period of exposure has been previously shown to induce both early (EAR) and late (LAR) airway responses in this species (Coyle et al., 1989; Coyle, Page, Atkinson, Flanagan, & Metzger, 1990).

2.5. Bronchoalveolar lavage

Following completion of pulmonary function measurements, a polyethylene cannula (outer diameter 1.34 mm) was passed down the endotracheal tube until just above the carina. Sterile saline (5 ml) was instilled into, and immediately aspirated from, the lungs under vacuum and collected in a polystyrene tube and kept on ice. Total cell counts from the bronchoalveolar lavage (BAL) were determined under light microscopy using an improved Neubauer haemocytometer. For differential cell counts, 5–7 drops of BAL fluid were used for centrifugation (Shandon cytospin 2) and the cells were stained with Lendrum's stain (Lendrum, 1944). A total of 200 cells were counted under light microscopy and classified as neutrophils, eosinophils or mononuclear cells, based on standard morphological criteria.

2.6. Measurements of cutaneous sensitivity to antigen

Direct skin tests were performed in immunised rabbits following completion of lung function measurements. Rabbits were anaesthetised with an intravenous (i.v.) injection of sodium pentobarbitone (30 mg/kg), administered via an indwelling cannula (23 gauge Butterfly; Abbot Ireland Ltd, Sligo, Ireland) in the marginal ear vein. The dorsal skin hair was closely clipped and a balanced site pattern marked either side of the midline of the animal, according to a Latin square design. Evans blue dye (10 mg/kg; 2.5% in saline) was administered i.v. to aid visualisation of cutaneous inflammatory responses. Ten minutes later, serial twofold dilutions of antigen or PBS (0.1 ml) as control were injected intradermally (i.d.) in four replicates. The rabbits were euthanised 60 min after these i.d. injections with an overdose of sodium pentobarbitone (60 mg/kg; i.v.) and the back skin was removed. The results are expressed as the titration end point, which was taken as the concentration of antigen that caused a greater response in at least two replicates than that of the control when viewed from the underside. The concentration of antigen (*Alternaria tenuis*) is expressed as Protein Nitrogen units (PNU) units per ml.

2.7. Passive cutaneous anaphylaxis reactions

An index of the serum IgE from immunised rabbits was obtained by titration of immune sera samples against a non-immune serum in the passive cutaneous anaphylaxis (PCA) test. Serum was prepared from 10 ml blood obtained by cardiac puncture following an overdose of pentobarbitone (60 mg/kg; i.v.). Recipient naïve male NZW rabbits (2–3 kg) were anaesthetised with fentanyl citrate (0.0125 mg/kg, i.m.) and fluanisone (4 mg/kg, i.m.) and the dorsal hair closely clipped. A pattern of six blocks with 6 sites for 36 i.d. injections was made on the dorsal skin, either side of the midline, to allow for a latin square design of the experiments. Serial two-fold dilutions, between 1:1–1:64 in phosphate buffered saline (PBS) were made of six test sera per experiment and injected (0.1 ml; i.d.) into the recipient animals. After a 72 h fixation

period, the recipient animals were anaesthetised with sodium pentobarbitone (30 mg/kg; i.v.) via an indwelling cannula (23 gauge Butterfly) in the marginal ear vein. Evans blue dye (10 mg/ml) was administered i.v. followed 10 min later by i.v. antigen (*Alternaria tenuis* extract; 7500 PNU/kg). Thirty minutes after antigen challenge the rabbits were euthanised with an overdose of sodium pentobarbitone (60 mg/kg; i.v.) and the back skin removed. The titration end point was taken as the highest dilution at which the test serum caused a greater blueing response compared with the control in at least two recipient rabbits when the skin was viewed from the underside, i.e. a reciprocal of the dilution factor of neat immune serum. If the positive responses were present at the 1:64 dilution, the serum was tested a second time at dilutions between 1:16 and 1:512.

2.8. Statistical analysis

Airway responsiveness was expressed as the geometric mean $\pm$ standard error of the mean. Values for R_L , PC_{50} , and C_{dyn} , PC_{35} were $\log_{10}$ transformed before statistical analysis was performed with an ANOVA and differences between means evaluated by Tukey multiple comparison test or Student's paired *t*-test where applicable. Nonparametric tests were used in the analysis of eosinophil cell data. PCA titres (given as the reciprocal of the titration end point) and direct skin test titres (given as the lowest concentration of antigen giving a response) are presented as the median together with the range and were compared using the Mann–Whitney test. Mean values were considered significant at $P < 0.05$. Data presented in this paper are a collation of the work performed in allergic rabbits by different researchers over a number of years.

2.9. Reagents and drugs

Histamine diphosphate, methacholine hydrochloride, sodium pentobarbitone, hematoxylin, sterile Dulbecco's phosphate buffered saline (PBS), Evan's blue dye chromotrope 2R and ketamine hydrochloride, were obtained from Sigma (UK). Sterile 0.9% saline was from Baxter Healthcare (UK). Diazepam was from Roche Products Ltd. (U.K.). Hypnorm™ (a mixture of fentanyl citrate, 0.315 mg/ml and fluanisone 10 mg/ml) was supplied by Janssen Pharmaceutical Ltd., (UK). Xylazine was from Veterinary Drug Co (U.K.). *Alternaria tenuis* was from Greer laboratories Inc (Lenoir, NC, USA). Aluminium hydroxide gel was from FSA laboratories (Loughborough, UK).

3. Results

3.1. Baseline airway responses

The variation in basal airway responses of sensitised and control animals to histamine or methacholine is highlighted in Table 1. There

Table 1
Baseline airway responses.

	Saline	Sham	Immunised
<i>Methacholine</i>			
R_L , PC_{50}	3.1 (2.3–4.1)	3.7 (2.6–5.2)	2.7 (2.0–3.6)
C_{dyn} , PC_{35}	2.3 (1.8–3.0)	2.8 (2.1–3.8)	3.2 (2.4–4.3)
N	61	42	46
<i>Histamine</i>			
R_L , PC_{50}	9.3 (6.4–13.4)	3.7 (2.6–5.2)*	6.4 (5.4–7.6)
C_{dyn} , PC_{35}	6.4 (4.6–8.8)	2.8 (2.1–3.8)*	5.0 (4.2–6.0)
N	51	42	222

* $P < 0.05$ cf saline and immunised groups (Tukey's post hoc test).

Airway responses are represented as geometric mean and 95% confidence interval for R_L , PC_{50} and C_{dyn} , PC_{35} . N represents the total number of animals studied.

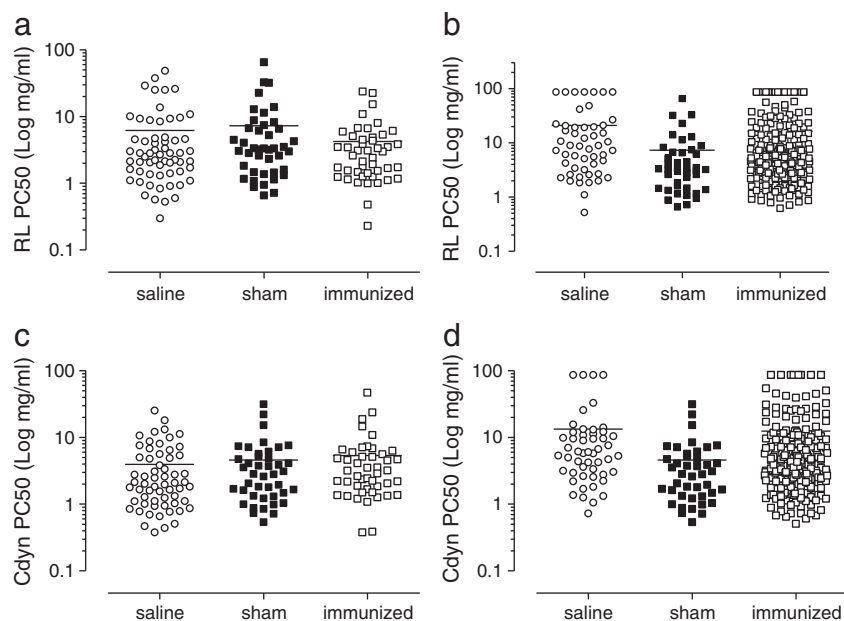


Fig. 1. Baseline airway responsiveness to methacholine (a, c) and histamine (b, d) expressed either as R_L PC₅₀ (a, b) or Cdyn PC₃₅ (c, d) in saline ($n = 61, 51$ methacholine, histamine, respectively), sham ($n = 42, 42$) and immunised ($n = 46, 222$) groups. $P < 0.05$ for histamine R_L PC₅₀ and Cdyn PC₃₅ value between saline and sham group and between sham and immunised groups.

was no significant difference in baseline airways responsiveness to aerosolised methacholine, as measured by R_L PC₅₀ and Cdyn PC₃₅, between saline, sham and immunised groups (Fig. 1). However, a one-way ANOVA of log transformed data for saline, sham and immunised groups revealed a significant difference in baseline sensitivity for

histamine as measured by R_L PC₅₀ and Cdyn PC₃₅ ($P = 0.0028$ and $P = 0.0035$ respectively; Table 1, Fig. 1). A further subgroup analysis based on littermate controls as a factor revealed no significant difference in airway sensitivity between saline, sham or immunised groups for histamine.

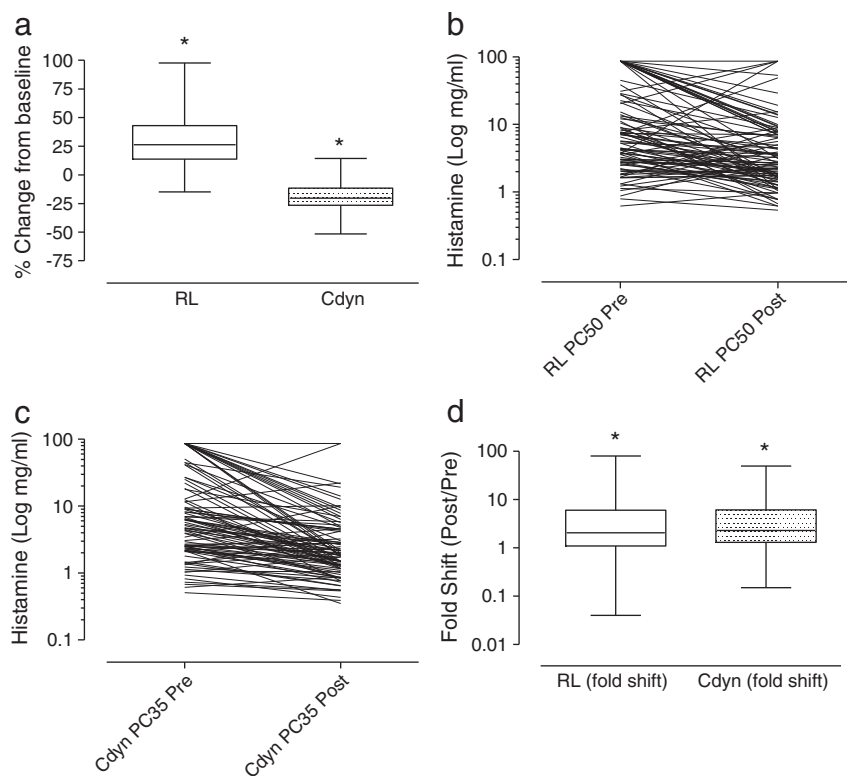


Fig. 2. Antigen induced responses in allergic rabbits. Allergen challenge caused a significant bronchoconstriction as evident by (a) change in baseline R_L and Cdyn ($n = 92$); and increase in airways responsiveness to histamine as assessed by (b) change in R_L PC₅₀; (c) increase change in Cdyn PC₅₀ and (d) post/pre change in airways responsiveness in terms of R_L and Cdyn. Significant change in the magnitude of the variables compared with control ($*P < 0.05$).

3.2. Airways response to acute allergen challenge

Acute challenge with aerosolised allergen resulted in an acute bronchoconstriction as measured by a significant increase in baseline R_L and fall in Cdyn (Fig. 2a). Change in airway sensitivity to histamine was measured following allergen challenge in 92 rabbits and the individual changes are shown (Fig. 2b, c). There was a significant increase in airway sensitivity (mean change, [95% Confidence interval]) to histamine as measured by changes in R_L PC50 (5 fold (Harrison et al., 2008; Pitchford et al., 2004; Persson, 2002; Pinckard et al., 1977; Shampain et al., 1982), $n = 92$) and Cdyn PC35 (6 fold [4–8], $n = 92$) (Fig. 2d).

3.3. Airway inflammation

Prior to allergen challenge the predominant cell types in lavage fluid were mononuclear cells (mean, SD; $96 \pm 6\%$ $N = 57$), neutrophils ($3.6 \pm 6.3\%$) and eosinophils ($0.1 \pm 0.4\%$) with a total cell count of $67 \pm 85 \times 10^4$ cells/ml ($n = 57$). Twenty-four hours after allergen challenge, there was a significant increase (mean change, 95% Confidence interval) in the recruitment of neutrophils ($12.3 [7.3–17.3] \times 10^4$ cells/ml, $P < 0.0001$) and eosinophils ($4.3 [3.2–5.4] \times 10^4$ cells/ml, $P < 0.0001$) to the lung which represented an 18% and 6% increase, respectively (Fig. 3).

3.4. Skin titre responsiveness to allergen and PCA reactions

The direct skin titre analysis (median, 25% and 75% percentile) showed a significant difference between sham (7.8 (0.75–312.5); $N = 26$) and immunised (0.125 (0.0625–0.375); $N = 65$) animals. There was also a significant difference in the PCA titre between sham (0 (0–0); $N = 41$) and immunised (4 (0.67–13.33); $N = 177$) animals. These differences were statistically significant ($P < 0.0001$) between sham and immunised groups (Fig. 4a, b).

3.5. Correlations

Various analyses were undertaken to determine whether there was any significant correlation between the variables measured (BHR, eosinophil cell number, PCA and skin titres). There was a significant correlation between skin titre ($P = 0.006$, Fig. 4c), PCA ($P = 0.0005$, Fig. 4d) and eosinophil recruitment to the lung 24 h following allergen challenge. There was a negative correlation between PCA and skin titre (Fig. 4) consistent with a lower dose of antigen required to elicit a skin response which correlates to a smaller sera dilution in the PCA reaction (Fig. 4e).

There was no significant correlation between allergen induced eosinophil recruitment (Fig. 5a, b) or PCA (Fig. 5c, d) versus antigen

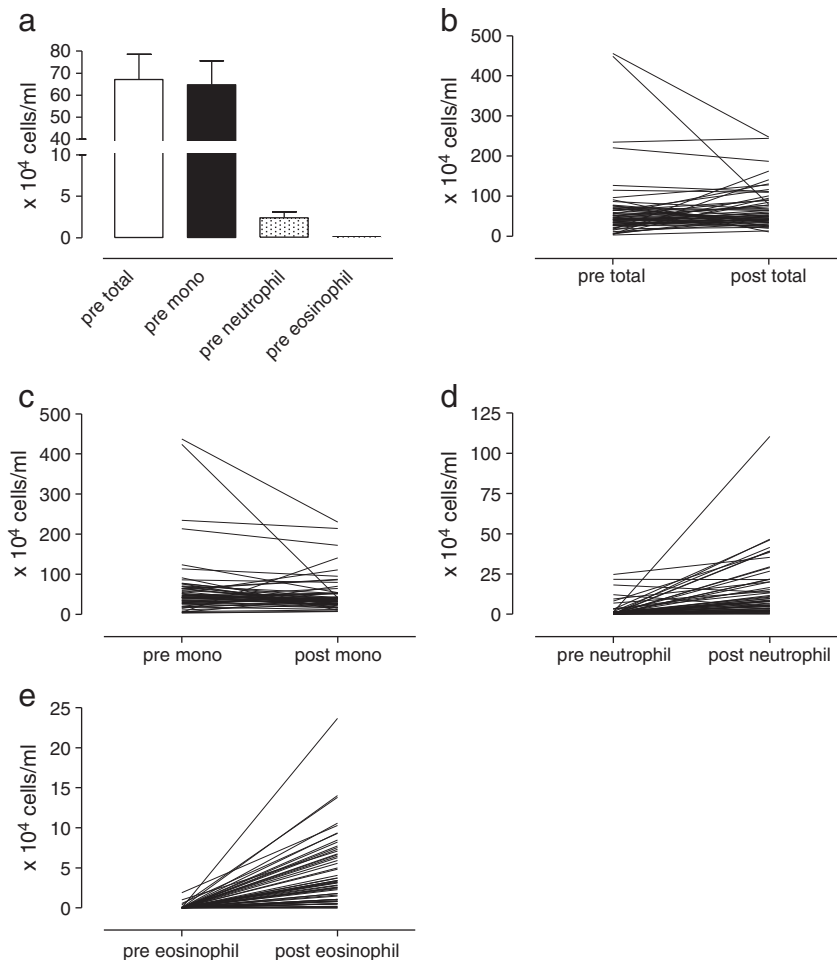


Fig. 3. Allergen-induced changes in airway inflammation. Total and differential number of cells in bronchoalveolar lavage fluid 24 h before antigen challenge (a). Total cell (b), mononuclear cell (c), neutrophil (d) and eosinophil (e) cell number 24 h before and then 24 h following antigen challenge in 57 rabbits. * $P < 0.05$ for total cells, neutrophils (log transformed, Student's paired t -test) and eosinophils (Wilcoxon paired t -test).

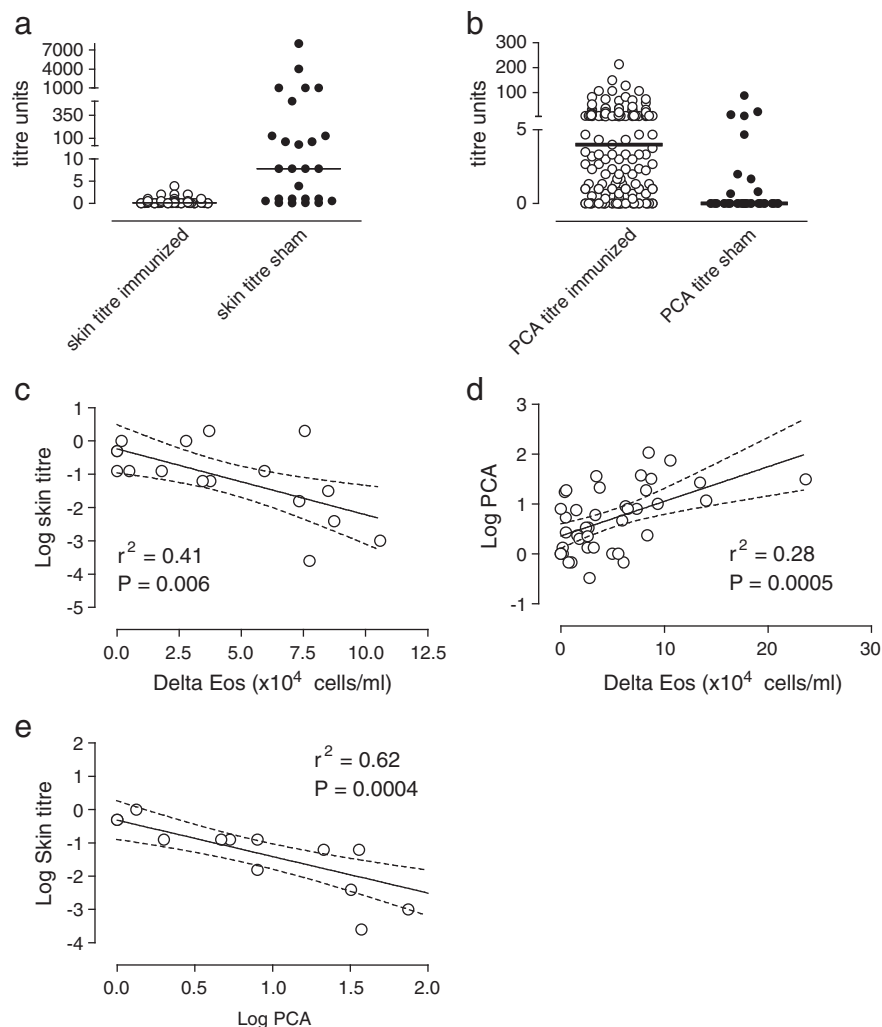


Fig. 4. Skin titre (a) and passive cutaneous anaphylactic (PCA) response (b) in sham ($n = 26, 41$ respectively) and immunised ($n = 65, 177$ respectively) rabbits. Line represents median (a, b) and the number of animals exhibiting zero skin titre units was 36 and 32, in the immunised and sham groups, respectively (b). There was a significant correlation between (c) skin titre ($n = 17$) and (d) PCA ($n = 40$) versus allergen induced increase in BAL eosinophils. (e) There was an inverse relationship between skin response and PCA ($n = 15$). Hence, the greatest PCA reaction was negatively correlated with more sensitive skin response to direct allergen injection.

induced BHR, using histamine R_L PC50 or Cdyn PC35 as measures of airway responsiveness.

There was no correlation between baseline PCA titre and baseline airway responsiveness to either histamine or methacholine, when measured as R_L or Cdyn (Fig. 6). Similarly, there was no relationship between skin titre and baseline airway responsiveness to either histamine or methacholine, when measured as R_L or Cdyn (Fig. 7). However, there were fewer animals included in this part of the study as fewer animals underwent a skin titre assessment.

4. Discussion

Animal models can be useful in investigating airway disease. The neonatally immunised rabbit has been shown to have many similarities to human subjects with asthma. Recording lung function in a minimally-invasive way allows repeated measurements to be made in each rabbit, and each animal may be used as its own control and animals can be studied for up to 1 year (Minshall et al., 1993), something which is technically challenging in smaller species.

In our work, we have demonstrated a wide variation in baseline airways responsiveness in allergic rabbits when using histamine or methacholine as a direct bronchoconstrictor stimulus. Moreover, in a

large population of allergic rabbits, this baseline responsiveness was not statistically different from sham littermate controls. However, allergen provoked an anticipated increase in airways obstruction, airways inflammation and exacerbations of airway responses to direct acting spasmogens. These results are comparable to those seen in man. Thus, in normal subjects, a 30-fold variation in airway responsiveness to methacholine has been observed (Jubber, Foster, Hassan, Carpenter, & Small, 1993). PC₂₀ values to both histamine and methacholine were shown to occur over a wide range of concentrations (0.062–4 mg/ml) (Hargreave et al., 1981). It has also been suggested that measuring the slope of the curve may also be beneficial (Orehek, Gayard, Grimaud, & Charpin, 1977), although there are controversies as to whether the slope is different in asthmatics and non-asthmatics (De Meer, Heederik, & Postma, 2002; Malo, Cartier, Pineau, Gagnon, & Martin, 1985). The wide variation in responsiveness to histamine and methacholine may be due to external factors, including previous allergen exposure, medication use, as well as technical factors such as nebuliser output, volume and speed of inspiration and method of exposure.

We have previously shown in a small cohort of animals a greater sensitivity of allergic rabbits to histamine than littermate controls (Minshall et al., 1993). However, this difference did not translate to a larger population as shown in the current study, although it must be

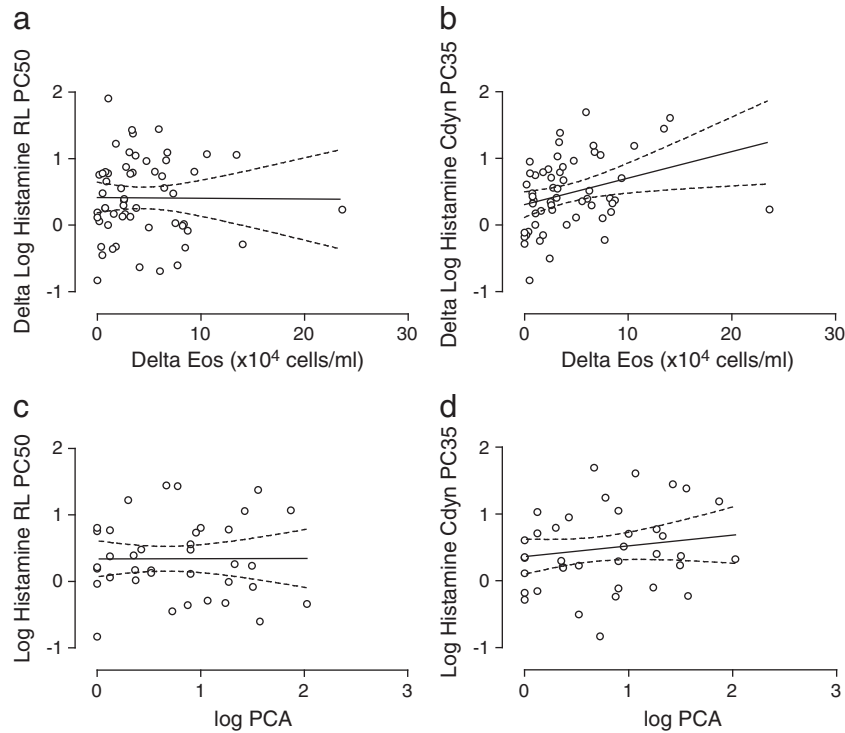


Fig. 5. Correlations post antigen challenge. There was an absence of correlation between the increase in airways responsiveness to histamine as assessed by changes in (a) RL PC50 ($n = 57$) and (b) Cdyn PC35 ($n = 57$) versus the increase in eosinophil number in lavage fluid. There was also an absence of a correlation between skin PCA reactions and (c) histamine RL PC50 ($n = 41$) or (d) histamine Cdyn PC35 ($n = 41$).

pointed out that the rabbits described in this study were not formally set up to investigate this difference. We undertook a sub-group analysis of the baseline airways responsiveness data using littermate as a fixed factor in order to minimise the variance associated with undertaking these measurements by different investigators over time, but even blocking for litter did not change the overall conclusion of the larger

analysis, namely that baseline airway sensitivity to histamine was not increased. Similar discrepancies can arise in clinical studies. For example, many studies purport to show an exacerbation of BHR to methacholine 24 h following allergen challenge in mild asthmatics (Cockcroft et al., 2005), although this is not always the case (Ward, McKenniff, Evans, Page, & Costello, 1994).

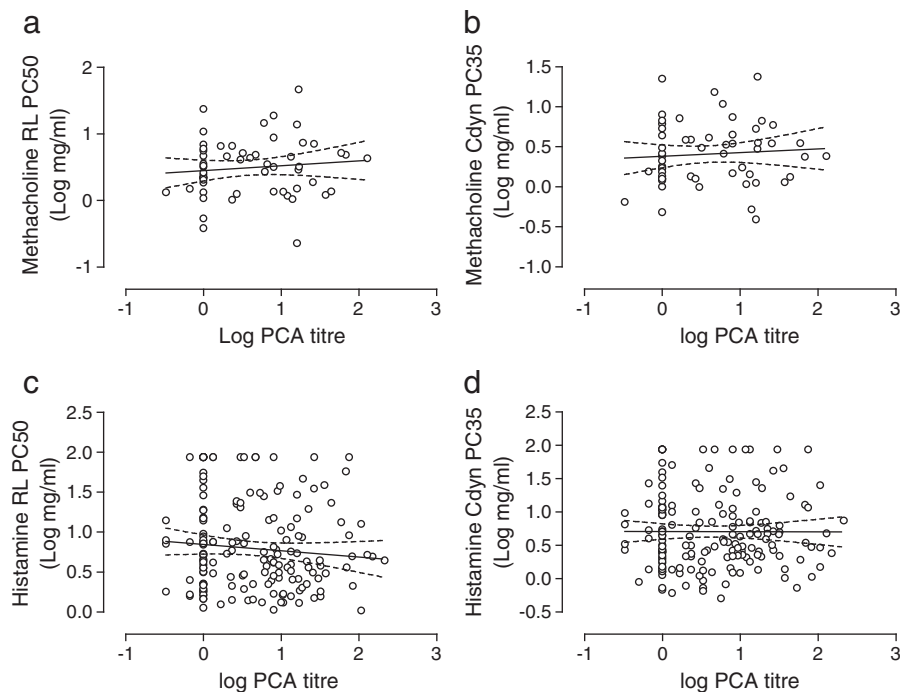


Fig. 6. Correlation analysis of baseline methacholine (a, b) or histamine airway sensitivity (RL PC50, Cdyn PC35 respectively) and PCA. There was an absence of correlation between baseline airway sensitivity to methacholine (a, b; $n = 57$) and histamine (c, d; $n = 173$) and PCA reactions in the skin of allergic rabbits.

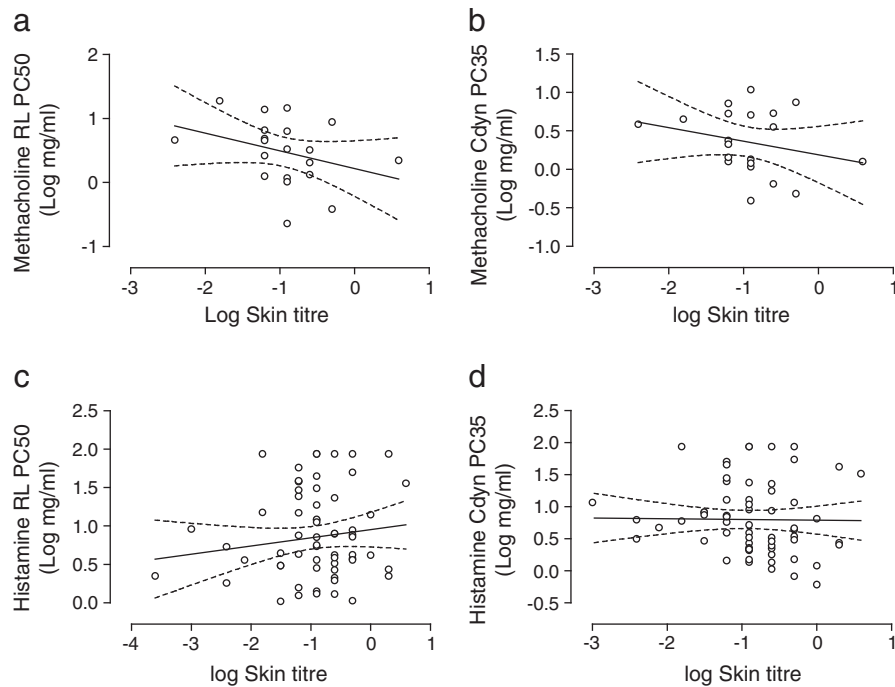


Fig. 7. Correlation analysis of methacholine (a, b) or histamine airway sensitivity (RL PC50, Cdyn PC35 respectively) and skin titre. There was an absence of correlation between baseline airway sensitivity to methacholine (a, b; $n = 20$) and histamine (c, d; $n = 65$) and PCA reactions in the skin of allergic rabbits.

Allergen challenge caused significant bronchoconstriction and BHR to histamine and methacholine in our rabbits. As we did not measure allergen PC₂₀, we were unable to analyse any correlation between skin reactivity and EAR in the airways of allergic rabbits. Whilst early research showed that there was a weak correlation between cutaneous and airway responses to allergen (Aas, 1975), other research has shown that skin sensitivity to allergen did not accurately predict airway responsiveness to inhaled allergen (Bowton, Fasano, & Bass, 1998). However, more recently, subjects with moderate to severe atopic dermatitis that were skin test positive to allergen were shown to have a higher risk of BHR (Kyllönen et al., 2006). Other studies have reported that the airway response to allergen is related to both the degree of airway responsiveness to histamine or methacholine and the level of IgE (as assessed by the skin test) (Bryant et al., 1975; Cockcroft et al., 1979; Howarth et al., 1985; Killian, Cockcroft, Hargreave, & Dolovich, 1976).

The lack of a correlation between eosinophil number and BHR at the 24 h time point could simply reflect differences in the time course between eosinophil recruitment and the expression of BHR. Hence, a correlation might be evident at earlier time points. However, this seems less likely in view of the retention of eosinophils within airway tissue. Indeed, clinical studies have often reported a similar degree of eosinophil number at 7 and 24 h post antigen challenge in asthmatic subjects (Gauvreau et al., 2011). Consequently, we would anticipate that sampling the lung at 24 h would be adequate to define a relationship between cell number and changes in airway sensitivity to spasmogens. We acknowledge that a measure of eosinophil activation might be a more sensitive indicator of changes in lung function. However, because of the lack of biological tools in the rabbit, we were unable to quantify activation status in our experiments. The release of eosinophil peroxidase or retention of this enzyme by eosinophils could be utilised as a marker of eosinophil activation (Hughes et al., 1996), although this has not been validated in our model. Nonetheless, our results are consistent with a growing number of clinical observations that eosinophils and bronchial hyperresponsiveness may be dissociated (Spina & Page, 2002).

Our study shows that there is a correlation between skin reactivity and airway eosinophilia, which supports a weak correlation between skin

responses and airway eosinophilia reported in mild allergic asthmatics (Boulay et al., 2003). A correlation between hyperresponsiveness to histamine or methacholine, and allergen skin sensitivity has previously been demonstrated in humans (Cockcroft et al., 1979; Cockcroft et al., 1987; Cockcroft et al., 2005). Furthermore, the histamine/methacholine PC₂₀ and allergen PC₂₀ were also fairly reproducible. The skin test end point and histamine/methacholine PC₂₀ allow a fair prediction of allergen PC₂₀. Another study showed that asthmatic subjects with comparable antigen skin test reactivity exhibited variability in the quantity of mediators that could be released following allergen challenge (Bedard, Atkins, & Zweiman, 1983). However, the magnitude of the mediator release in the skin was related to the antigen skin-test sensitivity of the host and the dose of antigen.

In our experience of using allergic rabbits, we have failed to record any correlation between skin sensitivity or immune status (PCA titre) and airway responsiveness to inhaled histamine, methacholine or allergen, i.e. skin test reactivity is not a surrogate marker of airways responsiveness. However, skin reactivity did predict airway inflammation as assessed by measurements of eosinophil recruitment to the lung, further highlighting the dissociation between airway inflammation and BHR (Spina & Page, 2002).

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