



Establishing the phenotype in novel acute and chronic murine models of allergic asthma

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Abstract

Allergic asthma is a chronic disease of the airways, with superimposed acute inflammatory episodes which correspond to exacerbations of asthma. Two novel models of allergic asthma have been developed in mice receiving the same allergen sensitisation, but with acute or chronic allergen exposures, the latter to mimic the human situation more closely. Ovalbumin-sensitised mice were challenged by ovalbumin inhalation twice on the same day for the acute model, and 18 times over a period of 6 weeks for the chronic model. Lung function was monitored in conscious, unrestrained mice immediately after the last challenge for up to 12 h. Airway responsiveness to inhaled methacholine and serum antibody levels were determined 24 h after challenge. Bronchoalveolar inflammatory cell recruitment was determined at 2 or 24 h.

Acute and chronically treated mice had similar early and late asthmatic responses peaking at 2 h and 7–8 h, respectively. IgE and IgG antibody levels, compared with naïve mice, and eosinophil infiltration, compared with naïve and saline challenge, were elevated. Airway hyperresponsiveness to methacholine was observed 24 h after challenge in both models. The acute model had higher levels of eosinophilia, whereas the chronic model showed hyperresponsiveness to lower doses of methacholine and had higher levels of total IgE and ovalbumin-specific IgG antibodies. Both novel murine models of allergic asthma bear a close resemblance to human asthma, each offering particular advantages for studying the mechanisms underlying asthma and for evaluating existing and novel therapeutic agents.

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1. Introduction

Ascertaining whether asthma is present in humans is usually based on the presence of clinical symptoms such as episodic cough, wheezing, breathlessness and chest tightness and on a characteristic history and variability in lung function measured

with spirometry or peak-flow measurements [1]. However, a diagnosis based largely on consistent symptoms and home peak-flow reading is often inaccurate due to poor patient technique and compliance, requiring further investigation [2]. *In vivo* animal models of the disease help to understand the pathogenesis of asthma permitting the study of parameters that would be difficult to assess in humans and have ethical problems. An ideal animal model should resemble the major features of human asthma. Asthma is a chronic disease of the airways characterised by the chronic presence of inflammatory cells such as eosinophils, mast cells and T lymphocytes, with superimposed acute inflammatory episodes which correspond to exacerbations of asthma [3–5]. In addition, asthmatic airways undergo chronic airway remodelling that, together with the accumulation of inflammatory cells, may contribute to the development of airway hyperresponsiveness (AHR). This consists of an exaggerated response of the airways to a variety of non-specific stimuli contributing to exacerbations of asthma [6,7].

Allergic asthma is the most prevalent type of asthma affecting two thirds of the total asthmatic population and 80% of asthmatic children and adolescents [8,9]. The acute asthmatic response after antigen inhalation in sensitised atopic asthmatic patients results in an early asthmatic response (EAR) which develops immediately after challenge reaching a maximal bronchoconstriction between 15 and 30 min and generally resolving within 1–3 h [10,11]. The EAR is the result of an immediate IgE-dependent type I hypersensitivity reaction driven by the activation of mast cells, alveolar macrophages, dendritic cells and airway epithelial cells among others [12]. These cells release mediators such as histamine, prostaglandins, leukotrienes and thromboxanes that are involved in the bronchoconstriction, mucus secretion and microvascular leakage observed during the EAR. In addition, these mediators release cytokines and chemotactic factors that are essential for the recruitment and activation of further inflammatory cells involved in the development of the late asthmatic response (LAR) in some patients [10,12]. Depending on the intensity and duration of the stimuli, approximately 60% of asthmatic patients are dual responders eliciting two temporally distinct bronchoconstrictor responses [13,14]. This LAR is characterised by a slowly progressive and persistent bronchoconstriction that begins 3–4 h after allergen provocation, peaks between 6 and 12 h and generally resolves within 24 h [13]. The late phase response develops as a result of activation of inflammatory cells which release pro-inflammatory mediators contributing to the development of allergen-induced AHR [11] and perpetuating the asthmatic inflammatory response [12] typical of chronic human asthma.

Animal models have been developed to study the pathogenesis of asthma. Guinea-pig models of asthma can provide the essential hallmarks of asthma, including dual bronchoconstrictor responses (EAR and LAR) [15–18]. However, asthma models in mice are potentially more useful due to the fact that their immune system has been extensively characterised, genetically modified animals (knockout, transgenic and immunodeficient mice) are available and a wide range of species-specific reagents can be obtained [9,19]. Different protocols have been employed for induction of allergic asthma in mice, and can be differentially classified into acute or chronic models depending on the

number of exposures to the allergen. Acute models of allergic asthma expose the animals to the allergen over a relatively short period of time, obtaining an asthma-like phenotype that resembles human asthmatic airways during exacerbations of the disease. On the other hand, chronic models expose the animals to the allergen over longer periods to mimic the recurrent long-term exposure to low concentrations of allergen experienced by people with asthma [9]. In this report, we compare the effects of acute and chronic exposure to allergen on asthmatic hallmarks developed in two novel murine models of allergic asthma receiving the same sensitisation. While there have been a number of studies using acute and chronic exposures of mice to allergen, this study describes for the first time a chronic murine model of allergic asthma that displays both EAR and LAR, alongside measures of AHR, serum antibody levels and lung inflammatory cell counts.

2. Methods

2.1. Sensitisation and challenge

Male BALB/c mice weighing 20–25 g were maintained under conventional animal housing conditions receiving food and drinking water *ad libitum*. All studies complied with the guidelines for the care and use of laboratory animals according to the Animals (Scientific Procedures) Act 1986. All mice except the naïve animals were sensitised on days 0 and 5 by i.p. injection of ovalbumin (OVA, 100 µg/mouse) and aluminium hydroxide (10%, 50 mg/mouse) in phosphate-buffered saline (PBS). Twelve days after the last injection, mice in the acute group (Ac O/O) were challenged by inhalation of a 0.5% (w/v) OVA aerosol in 0.9% sodium chloride solution (saline) twice on the same day, 4 h apart. Mice in the chronic group (Ch O/O) were challenged over a period of 6 weeks by inhalation of a 2% (w/v) OVA aerosol in saline for 30 min/day on 3 days/week (18 challenges). The choice of the sensitisation protocol was based on preliminary studies in our laboratories involving a fully characterised asthmatic model in guinea-pigs [20], whereas the choice of challenge protocols was based on successful models described in the literature for acute [21] and chronic [22] models. We used a higher concentration of OVA in the chronic model because it is known that tolerance can develop to repeated exposures to inhaled antigen in both humans [23] and mice [24]. The aerosols were delivered by a PulmoStar nebuliser (Sunrise Medical, Stourbridge, U.K.) to a polystyrene exposure chamber (14.5 cm×28.5 cm×15 cm) in which groups of mice were contained. Mice in control groups Ac O/S and Ch O/S were sensitised with OVA and aluminium hydroxide but challenged with saline for the same period of time as the respective group O/O. Naïve animals were not challenged.

2.2. Non-invasive determination of airway function

Airway function was measured in unrestrained, conscious mice by barometric plethysmography using a single chamber, whole-body plethysmography (WBP) system (Buxco Research Systems, Winchester, U.K.) according to the manufacturer's instructions. Enhanced pause (Penh) was used as a measure of airway responsiveness as described by Hamelmann et al. [25]. For determination of allergen-induced changes in airway function (EAR and LAR), Penh was assessed by placing the animals in the chamber singly for a period of 1 min. Penh values were measured before the challenge (baseline reading) and then at 0, 20, 40, 60, 90 and 120 min after the last OVA or saline challenge for all groups. Penh was then assessed every 60 min during the first 10 h after challenge with a final readout 24 h after the first challenge for the acute groups and 24 h after the last challenge for the chronic groups. Results are expressed as mean

values for percentage changes in Penh with respect to baseline $\pm$ standard error of the mean (S.E.M). The area under the curve (AUC) was calculated for each individual using the program GraphPad Prism and results expressed as mean $\pm$ S.E.M for each group. The AUC for the EAR was calculated between 0 and 4 h for the acute groups and 0 and 5 h for the chronic groups. The AUC for the LAR was calculated between 4 and 10 h for the acute groups and 6 and 9 h for the chronic groups.

In addition, airway responsiveness to inhaled methacholine was assessed 24 h after the first OVA or saline challenge in the acute groups or 24 h after the last OVA or saline challenge in the chronic groups. Baseline Penh was measured for 1 min before methacholine inhalation. Thereafter, Penh was assessed for 5 min after provocation with 10 or 30 mg/ml methacholine aerosol delivered into the recording chamber via an Aerogen nebuliser (Buxco Research Systems). Results are expressed as mean values for percentage changes in Penh with respect to baseline $\pm$ S.E.M.

2.3. Total IgE and OVA-specific IgG

Serum samples were collected from naïve animals and acute and chronically treated animals killed 2 or 24 h after the first OVA or saline challenge in the acute groups or 24 h after the last OVA or saline challenge in the chronic groups. Total IgE and OVA-specific IgG levels were determined in duplicate. Total IgE levels were measured using a direct sandwich Enzyme Linked Immunosorbent Assay (ELISA) [26]. Microplates were coated overnight at 4 °C with 10 μ g/ml goat anti-mouse IgE. Serum samples diluted in phosphate-buffered saline Tween (PBST) buffer (pH 7.4; 1.5 mM KH_2PO_4 ; 0.137 M NaCl; 2.7 mM KCl; 8 mM Na_2HPO_4 ; 0.05% (v/v) Tween-20) were incubated for 2 h at room temperature and then plates were treated with 1:1000 monoclonal rat anti-mouse IgE conjugated to horseradish peroxidase. After addition of enzyme substrate solution containing orthophenylene diamine (OPD), the absorbance at 490 nm was measured and the concentration of total IgE in the serum samples was calculated with reference to serial dilutions of standard mouse IgE isotype control. OVA-specific IgG antibodies were measured using an indirect ELISA. Microplates were coated overnight at 4 °C with 10 μ g/ml OVA and then incubated with serum samples diluted with PBST buffer followed by 1:2000 goat anti-mouse IgG conjugated to horseradish peroxidase. After addition of enzyme substrate solution containing OPD, the absorbance was determined at 450 nm and the concentration of OVA-IgG in the serum samples was calculated with reference to serial dilutions of standard mouse IgG antibody. Antibody levels in OVA-sensitised and challenged animals (Ac O/O and Ch O/O) were compared to OVA-sensitised mice challenged with saline (Ac O/S and Ch O/S) and naïve animals.

2.4. Cellular infiltration

Bronchoalveolar lavage (BAL) fluid was withdrawn from the lungs of naïve mice and mice killed 2 (acute) or 24 h after the first OVA or saline challenge in the acute groups or 24 h after the last OVA or saline challenge in the chronic groups after introducing 1 ml of 0.9% sodium chloride solution via the trachea three times. Smears of BAL cells were prepared for differential cell count by cytocentrifugation of 200 μ l samples (Cytospin, Shandon Scientific Ltd., Runcorn, U.K.) at 113 g for 7 min at room temperature. All slides were differentially stained with 0.15% Leishman's stain (eosin-polychrome methylene blue) in 100% methanol, pH 6.4–6.6. A minimum of 200 cells was counted under a light microscope at 1000 $\times$ magnification in two different slides per animal. Standard haemocytological procedures were used to identify four different types of leucocytes in the BAL fluid samples: lymphocytes, macrophages, eosinophils and neutrophils. Results are expressed as a percentage of the total number of cells counted. A total cell count was not possible because of the occasional presence of red blood cells in the BAL fluid.

2.5. Statistical analysis

Significant differences ($p < 0.05$) between two groups were calculated using an unpaired t -test (two tail p value), whereas statistical comparisons between more than two groups were calculated using one-way analysis of variance (ANOVA) followed by a Tukey–Kramer multiple comparison *post-hoc* test in GraphPad InStat v.3.06.

2.6. Materials

Antibodies employed for total IgE ELISAs were obtained from SouthernBiotech, (Cambridge Bioscience, Birmingham, U.K.). Standard mouse IgG antibody and acetyl- β -methylcholine chloride (methacholine chloride) were supplied by Sigma (Poole, U.K.), whereas goat anti-mouse IgE-HRP was supplied by Pierce (Perbio Science, Northumberland, U.K.). Aluminium hydroxide (RectapurTM) was obtained from

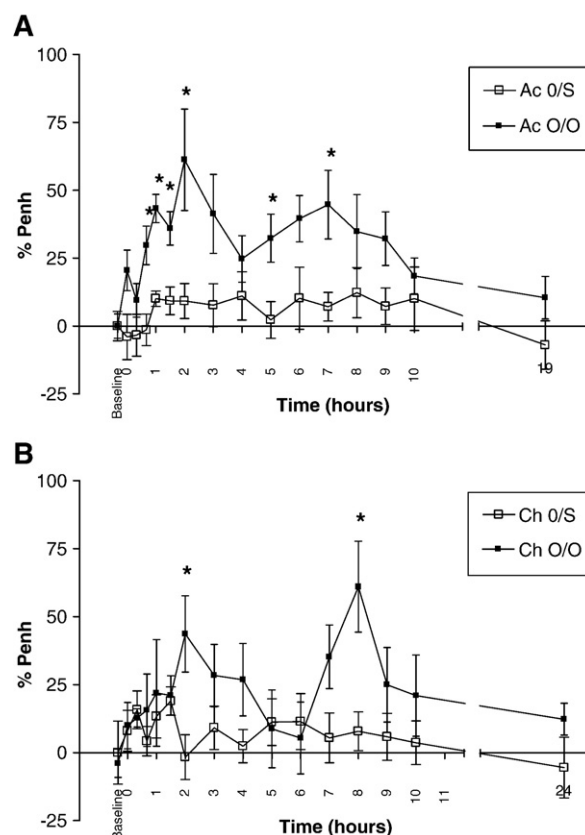


Figure 1 Early and late phase asthmatic responses in OVA-sensitised acute (A) or chronic (B) OVA-challenged mice (O/O) compared with OVA-sensitised acute (Ac) or chronic (Ch) saline-challenged mice as a control group (O/S). Changes in enhanced pause (Penh) were expressed as percentage of the baseline value obtained immediately before the first OVA or saline challenge for the acute groups or the last OVA or saline challenge for the chronic groups (mean $\pm$ S.E.M) ($n = 4-5$). The area under the curve (AUC) was calculated for each group for the EAR between 0 and 4 h for the acute groups and 0 and 5 h for the chronic groups. The AUC for the LAR was calculated between 4 and 10 h for the acute groups and 6 and 9 h for the chronic groups. Statistical differences between groups O/O and O/S were calculated for each time point ($*p < 0.05$) and for the AUC employing an unpaired t -test (two-tail p value). The AUC for groups O/O was significantly different from groups O/S in both the acute and chronic models, $p < 0.05$.

Merck Eurolab (Briare le Canal, France). Ovalbumin was supplied by BDH (Poole, U.K.). All other chemicals were obtained from Fisher Chemicals (Loughborough, U.K.) and were of Analar grade.

3. Results

3.1. Allergen-induced airways responses

The final inhalation challenge of OVA in acute and chronically OVA-exposed mice showed two temporally distinct bronchoconstrictor responses (Fig. 1A and B). Increases in Penh were observed immediately after OVA challenge, reaching a maximum 2 h later in both models. This phase was followed by a LAR with maximal Penh values at 7 h after OVA challenge in the acute model (Fig. 1A) and at 8 h in the chronic model (Fig. 1B). The Penh values at time points corresponding to the maximum EAR and LAR in both OVA-sensitised and challenged mice (O/O) were significantly greater than the saline-challenged groups (O/S). EAR and LAR showed similar patterns in both acute and chronic models and were not significantly different at any time point with the exception of 6 h after challenge ($p < 0.05$). Penh values were restored almost to baseline at 24 h after the first OVA or saline challenge in the acute groups or 24 h after the last OVA or saline challenge in the chronic groups. The AUCs for the EAR in both acute and chronically OVA-sensitised and challenged mice (O/O) were significantly different from the saline-challenged groups (O/S) (mean AUC for EAR: Ac O/O = 9961 ± 3101.2 ; Ac O/S = 2312.3 ± 1120.3 ; Ch O/O = 8385.3 ± 2488.5 ; Ch O/S = 2502.8 ± 936.8 ; $p < 0.05$). Significant differences were also observed between O/O and O/S groups for the AUCs during the LAR of acute and chronic animals (mean AUC for LAR: Ac O/O = 12318 ± 2969.6 ; Ac O/S = 4165.2 ± 1895.5 ; Ch O/O = 6841 ± 1947.8 ; Ch O/S = 2025.8 ± 701 ; $p < 0.05$).

3.2. Cellular infiltration and levels of total IgE and OVA-specific IgG

The percentages of eosinophils and neutrophils present in the BAL fluid samples from Ac O/O mice were significantly increased

compared with control and naïve groups 24 h after challenge (Fig. 2). In the chronic model, the percentage of lymphocytes, eosinophils and neutrophils were significantly increased compared with control and naïve animals (Fig. 2). This was accompanied in both models by a significant relative decrease in the percentage of macrophages compared to naïve animals (Ac O/O) or all other groups (Ch O/O) (Fig. 2). In BAL fluid samples taken 2 h after the last challenge from the Ac O/O group, significant percentage increases compared to naïve animals were seen for eosinophils and neutrophils (naïve: eosinophils 0.02 ± 0.02 , neutrophils 0 ± 0 ; Ac O/O: eosinophils 2.41 ± 0.75 , neutrophils 1.56 ± 0.71 ; $p < 0.05$).

The serum levels of total IgE and OVA-specific IgG in chronically OVA-sensitised and challenged mice (Ch O/O) were significantly increased compared to all other groups (Fig. 3B and D). The serum levels of total IgE and OVA-specific IgG in acutely OVA-sensitised and challenged mice (Ac O/O) were significantly increased compared to naïve animals (Fig. 3A and C). There was also an increase in OVA-specific IgG in sensitised animals after acute saline challenge compared to naïve animals (Fig. 3C) and increases in total IgE and OVA-specific IgG after chronic saline challenge (Ch O/S) compared with all other groups (Fig. 3B and D). Total IgE ($6.21 \pm 1.38 \mu\text{g/ml}$) and OVA-specific IgG ($64.36 \pm 13.35 \mu\text{g/ml}$) levels in serum from Ac O/O mice taken 2 h after the last challenge were also significantly increased compared to naïve animals (IgE $0.78 \pm 0.18 \mu\text{g/ml}$; IgG $0.73 \pm 0.33 \mu\text{g/ml}$; $p < 0.05$).

3.3. AHR to inhaled methacholine

24 h after the first OVA challenge of the acute OVA-sensitised mice there was a significantly greater bronchoconstriction after exposure to 30 mg/ml methacholine compared to naïve animals and the saline-challenged group (Fig. 4A). 30 mg/ml methacholine was not tested in the Ch O/O mice as preliminary experiments produced such a severe bronchoconstrictor response that it was not possible to test this dose in all animals. There was a significantly greater bronchoconstriction to the lower dose (10 mg/ml) of methacholine in the Ch O/O mice 24 h after the

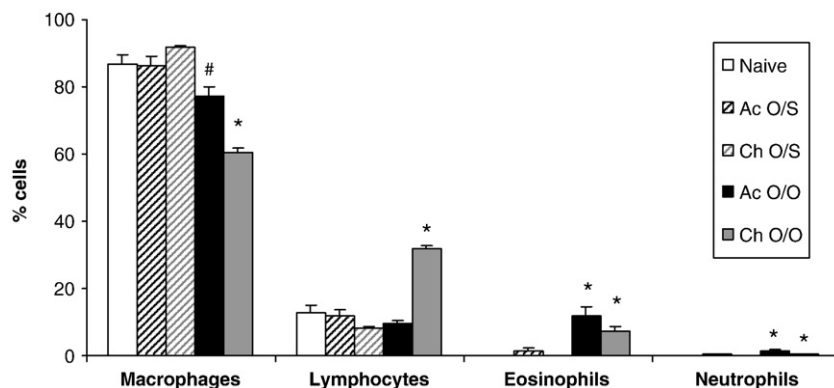


Figure 2 Cellular infiltration in BAL fluid samples from naïve mice, acute and chronic OVA-sensitised and challenged male mice (Ac O/O and Ch O/O) and acute and chronic OVA-sensitised but saline-challenged mice as a control group (Ac O/S and Ch O/S). Results are expressed as the percentage of macrophages, lymphocytes, eosinophils and neutrophils 24 h after the first challenge (acute) or last challenge (chronic) (mean $\pm$ S.E.M) ($n = 3-10$). Statistical differences within the same model were calculated for each cell type using one-way ANOVA with a Tukey–Kramer multiple comparisons post-hoc test. *Significantly different from all other groups; # significantly different from naïve; $p < 0.05$.

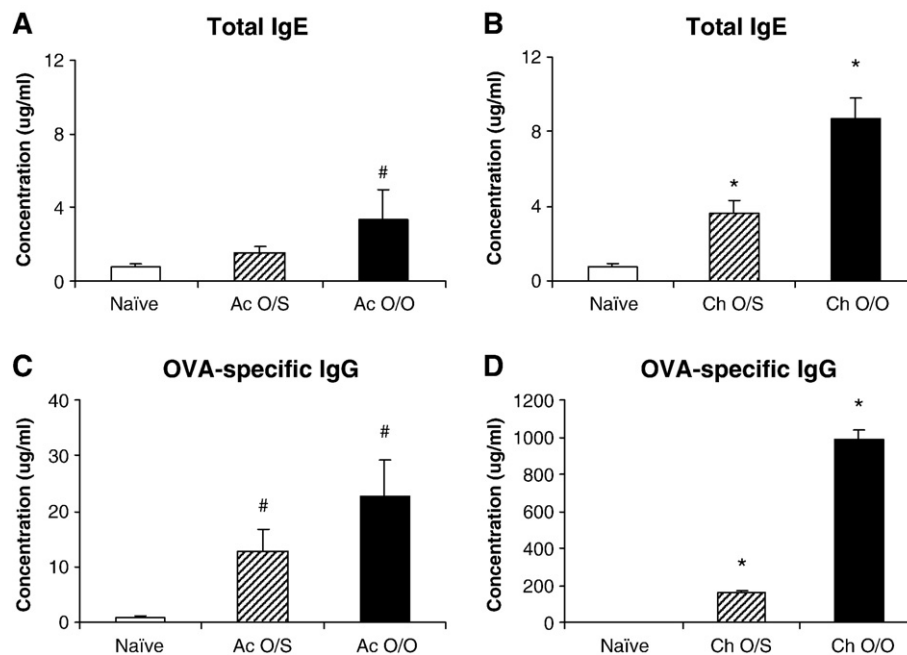


Figure 3 Serum levels of total IgE (A, B) and OVA-specific IgG (C, D) in naïve mice, acute (Ac) and chronic (Ch) OVA-sensitized and challenged mice (O/O) and acute and chronic OVA-sensitized but saline-challenged mice as a control group (O/S). Results are expressed as the concentration of antibody (µg/ml) 24 h after the first challenge (acute) or last challenge (chronic) (mean ± S.E.M) ($n=3-9$). Statistical differences were calculated using one-way ANOVA with a Tukey–Kramer multiple comparisons post-hoc test. *Significantly different from all other groups; # significantly different from naïve; $p<0.05$.

last OVA challenge compared to naïve animals and the saline-challenged group (Fig. 4B).

4. Discussion

4.1. Allergen-induced airways responses, cellular infiltration and levels of total IgE and OVA-specific IgG

Novel models of allergic asthma have been developed in male mice with the aim of reproducing the major features of the human condition in order to address both the acute and chronic condition of the airways during asthma. The asthmatic response after antigen inhalation in patients with allergic asthma results in an EAR due to an IgE-dependent type I hypersensitivity reaction [12]. This asthmatic feature was reproduced in both acute and chronic murine models of allergic asthma which showed an EAR commencing immediately after antigen challenge. In addition, approximately 60% of asthmatic patients are dual responders eliciting a LAR [13,14] characterised by a slowly progressive and persistent bronchoconstriction as a result of activation of inflammatory cells, mainly T-helper 2 cells and eosinophils [10]. The LAR was also seen in both the acute and chronic models. Moreover, both models showed eosinophilic inflammation and production of IgE and IgG antibodies indicative of a Th2 immunological pattern [27], a situation that mimics the human condition. Release of pro-inflammatory mediators during the LAR contributes to the development of allergen-induced airway hyperresponsiveness (AHR) [11] and perpetuates the asthmatic inflammatory response in human asthma [12]. This has been reproduced in the acute and

chronic murine models, which showed increased bronchoconstriction to inhaled methacholine 24 h after antigen challenge of OVA-sensitized mice. Therefore, the acute and chronic murine models of allergic asthma described here have been demonstrated to reproduce many of the important hallmarks of the human condition.

The two temporally distinct asthmatic responses have been previously reproduced in guinea-pig models of asthma, such as the acute model developed in our laboratories that closely mimics the situation in dual responder patients [28,29]. A biphasic response has also been demonstrated in acute murine models of allergic asthma [30–34], but has yet to be investigated in chronic models of asthma in mice. The time-course of the EAR and LAR described in the literature is very variable between acute models probably due to differences in the genetic background of the mouse strains and in the sensitisation and challenge protocols employed, although all protocols involved relatively short exposure to antigen. To our knowledge, this is the first time that two temporally distinct bronchoconstrictor responses have been demonstrated in a chronic murine model of asthma following the final allergen challenge of a series of chronic challenges. The final inhalation exposure to allergen of chronically sensitized mice (O/O) elicited an immediate asthmatic response that reached its maximum 2 h after challenge, followed by a LAR between 6 and 11 h. The EAR and LAR seem to be allergen-specific because they were absent in OVA-sensitized animals challenged with saline (O/S).

In our acute and chronic murine models of allergic asthma, the appearance of the LAR closely resembled that observed in human asthma. However, the time to peak of the EAR was delayed. Serum levels of total IgE and OVA-specific

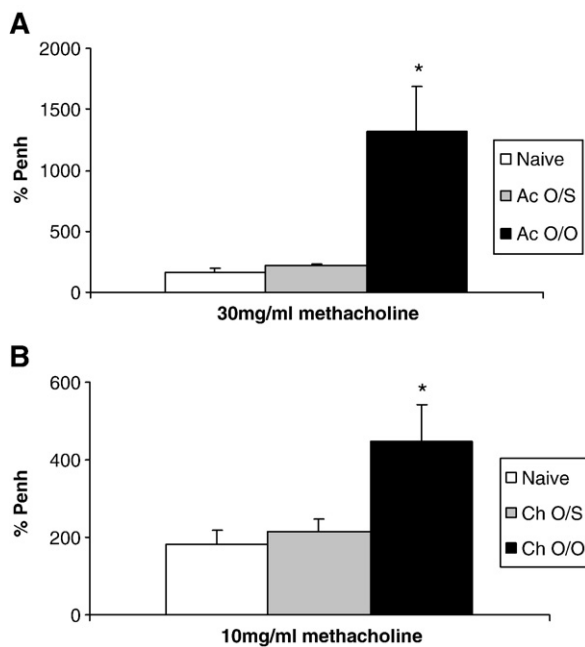


Figure 4 Bronchoconstriction to inhaled methacholine (10 or 30 mg/ml) in naïve mice and acute (Ac) and chronic (Ch) OVA-sensitised and challenged mice (O/O) and acute and chronic OVA-sensitised but saline-challenged mice as a control group (O/S) 24 h after the first challenge (acute) or last challenge (chronic). Peak changes in enhanced pause (Penh) after methacholine exposure were expressed as percentage of the baseline value prior to the methacholine provocation (mean $\pm$ S.E.M) ($n=5-6$). Statistical differences were calculated using one-way ANOVA with a Tukey–Kramer multiple comparisons post-hoc test. *Significantly different from naïve and control group O/S; $p<0.05$.

IgG were increased 24 h after challenge in both acute and chronic O/O mice compared to levels in naïve non-treated mice, demonstrating a normal immune response to common inhaled allergens. Both acute and chronic models showed an antibody response to the OVA sensitisation and challenge protocols, reaching significantly higher levels in the long-term model 24 h after challenge. Elevated levels of OVA-specific IgG antibodies in saline-challenged animals after OVA sensitisation alone (Ac O/S and Ch O/S) compared to naïve animals indicated the effectiveness of the initial sensitisation. The levels of OVA-specific IgG were very high in the chronic model (Ch O/O) compared with the acute form (Ac O/O) and were most likely due to the repeated stimulation of the immune system with each exposure to the allergen. There was also an increase in both IgE and OVA-specific IgG (Ch O/S) in the chronic saline-challenged animals compared to the acute group (Ac O/S) which is difficult to explain. Saline challenge may induce the immune system over the 6 week period of saline challenges. Serum levels of total IgE in O/O acute mice were already significantly elevated 2 h after the last challenge, the peak of the EAR. Therefore, the late appearance of the EAR is unlikely to be related to the lack of an IgE-dependent type I hypersensitivity reaction. The delay in EAR in our murine models is more likely to be due to a delay in the release of mediators that provoke mast cell degranulation.

Mast cell activation in the EAR provokes the induction of eosinophil infiltration and the appearance of AHR [11,35]. Dual responder asthmatic patients show an increase in eosinophil levels present in the sputum collected 24 h after allergen provocation [11]. This has been reproduced in our acute and chronic murine models of allergic asthma which showed airway eosinophilia in the BAL fluid 24 h after the last challenge. Even higher levels were seen 72 h later in the acute model [36]. Release of pro-inflammatory mediators from the cells recruited contributes to the development of AHR demonstrated in acute and chronic O/O mice as an increased bronchoconstrictor response to inhaled methacholine 24 h after challenge. Chronic O/O animals showed increased responsiveness to 10 mg/ml inhaled methacholine, whereas acute O/O mice needed a higher methacholine dose to reveal the AHR. The use of a new, highly efficient nebuliser that allows delivery of a measured dose in dry conditions [37] made it possible to obtain AHR when exposing the animals to lower doses of methacholine than have been employed by other investigators [25,38–40].

4.2. Measurement of Penh

Airway function was studied employing a whole-body plethysmograph that measures the difference between two changes in the chamber pressure: a reduction due to animal inspiration of air, and an increment due to the lungs expanding [37]. In normal conditions these flows largely cancel each other out. However, in conditions such as asthma the expiratory waveform created in the WBP after allergen challenge is indicative of effort exerted by the animal during expiration [37]. This has been measured employing the method described by Hamelmann et al. [25] to monitor the airway responses in conscious, spontaneously breathing O/O mice by barometric whole-body plethysmography, employing enhanced pause (Penh) as an index of airway resistance. Changes in the box pressure signal or Penh were demonstrated to be a valid indicator of AHR after allergic sensitisation in mice and to track the changes in the respiratory system caused by bronchoconstriction [25]. The validity of Penh as a marker of airway responsiveness has been questioned in the literature [41,42]. However, Penh has since been repeatedly used as an indicator of airway responsiveness to bronchoconstrictors in murine models of asthma due to the advantages of the barometric WBP as a non-invasive technique [38–40,43–49].

4.3. Choice of model

The acute and chronic models of allergic asthma described in this study reproduce important hallmarks of the human condition employing mice as the animal of choice. This has a number of advantages such as the availability of molecular probes and transgenic animals. These results raise the question as to which model is a better approximation of the human condition. The chronic murine model more closely resembles the long-term exposure to allergen suffered by asthmatic patients. Both models showed similar early and late asthmatic phases, and to our knowledge, this is the first chronic murine model of allergic asthma described in the literature that displays both phases. Chronically treated

animals showed higher levels of antibody and AHR to methacholine, whereas the acute model showed higher levels of eosinophil infiltration into the lungs. The choice of the animal model employed for investigation of asthma depends upon the hypothesis to be investigated. The novel murine models described here will be useful tools to address many of the questions raised in the pathogenesis and treatment of the human disease. The acute model would be more useful for the investigation of the inflammatory and functional changes associated with the first few days of the process and for more rapid screening, whereas the chronic model could be employed for investigating airway wall remodelling and subsequent airway function changes [9]. Indeed, airway wall remodelling including epithelial hypertrophy, goblet cell hyperplasia/metaplasia and subepithelial fibrosis has been demonstrated in chronic models employing similar challenge protocols to that described in this study [8]. This would suggest that remodelling had occurred in the chronically treated mice described here, although no histology was performed in the present studies. The close resemblance of these models to human asthma makes them of potential value for the study of the mechanisms underlying asthma and for testing new anti-asthma treatments. The reasons why some asthmatic patients show only EAR while the majority are dual responders are still unknown and remain of particular interest [50,51]. Our demonstration, for the first time, of both EAR and LAR in the chronic model will aid further investigation of the underlying differences that determine whether or not a LAR develops.

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