

Sequential Development of Airway Hyperresponsiveness and Acute Airway Obstruction in a Mouse Model of Allergic Inflammation

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Key Words

Plethysmography · Methacholine · Inflammation, allergic · Tidal breathing · Ovalbumin

Abstract

Background: Mouse models have been established mirroring key features of human bronchial asthma including airway hyperresponsiveness (AHR). Acute airway obstruction in response to an allergen challenge, however, remains to be demonstrated in these models. **Objective:** A mouse model of allergic lung inflammation was employed to analyze the development of specific (allergen-induced) and nonspecific (methacholine-induced) airway obstruction. **Methods:** Mice were sensitized to ovalbumin (OVA) and challenged with OVA aerosol twice each week during four weeks. Changes in lung functions were determined by noninvasive head-out body plethysmography. The development of acute airway obstruction

after OVA challenge and AHR after methacholine aerosol application were assessed by a decrease in the mid-expiratory flow rate (EF₅₀). **Results:** Two airway challenges were sufficient to induce AHR (5.7 vs. 15 mg/ml methacholine). Further OVA challenges reduced the baseline EF₅₀ from 1.85 to 1.20 ml/s (4th week) and induced acute airway obstruction. The OVA-induced obstruction was maximal in the 4th week (EF₅₀ = 0.91 ml/s). **Conclusion:** The development of acute airway obstruction in allergen-sensitized mice was demonstrated by means of head-out body plethysmography. In our model, AHR was observed before the development of airway obstruction.

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Introduction

Allergic bronchial asthma is characterized by an allergen-specific immune response with IgE antibodies, the presence of airway inflammation, acute airway obstruction and airway hyperresponsiveness (AHR) [1]. Measurements of AHR have been traditionally employed to

This work is dedicated to the 60th birthday of Prof. Dr. Eckart Kötting.

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identify individuals who have asthma or have a risk to develop it. Histamine and methacholine are used most frequently as provocation stimuli [2].

Since the pathogenesis of bronchial asthma is not fully understood, considerable interest in the development of animal models has emerged. In recent years, murine models were developed which exhibit several key features of human bronchial asthma. When allergen-sensitized mice are challenged with allergen via the airways, a TH2-type inflammation is elicited. This type of airway inflammation is defined by an influx of IL-4 and IL-5 producing T-cells and eosinophils [3, 4]. It is dependent on (1) IgE and B cells [5, 6], (2) CD4 T-cells [7], (3) IL-4 and IL-5 [8–11], (4) eosinophils, and (5) it can be transferred into naive mice using defined TCR-V β -expressing T-cell subsets [12]. On the other hand, inflammation and AHR are abrogated by IFN- γ , sIL-4R or allergen-specific immunotherapy, and CD8 T-cells [13–16].

Several methods have been used to study lung or airway smooth muscle function in mice. Methods widely used are the measurement of airway resistance following anesthesia and tracheal cannulation [17], electric field stimulation of tracheal smooth muscle [18], and whole-body plethysmography [19]. The development of AHR in sensitized and airway-challenged mice has been demonstrated with these methods. However, obstructive response data during challenge by allergens are lacking in these models. Technical reasons might account for this lack of data, since the above-mentioned methods do not allow monitoring of lung function during allergen challenge in spontaneously breathing animals. We therefore relied on head-out body plethysmography which has been used to assess AHR [20] and overcomes this problem. A well-established murine model with IgE response, airway inflammation and AHR [18] was used to assess whether or not an acute airway response can occur in sensitized mice during aerosol allergen challenge.

The head-out body plethysmograph has been developed to determine changes in tidal breathing during inhalation of toxic and pharmacological agents such as carbamylcholine [21, 22]. With this method, airflow during inspiration and expiration is measured continuously. Airflow at mid-tidal volume during expiration (EF₅₀) is measured for each breath and a decrease is taken as an index of airway obstruction. A decrease in EF₅₀ during carbamylcholine challenge via the airways was demonstrated to be correlated with an increase in lung resistance in mice [22] as measured by the classical method of Amdur and Mead [23]. Therefore, this parameter was used to assess AHR during methacholine challenge as well as to deter-

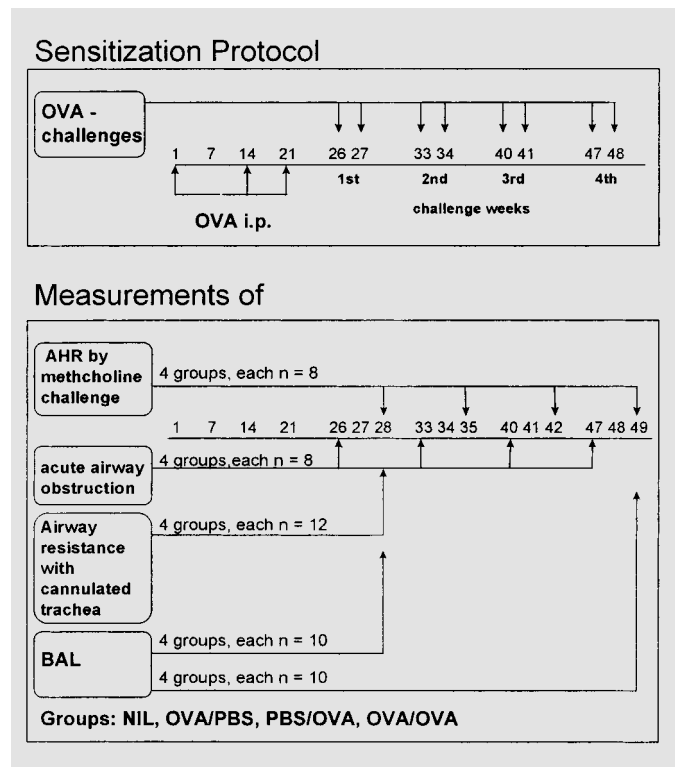


Fig. 1. Schematic drawing of the sensitization protocol. Numbers indicate days. For clarity, counting of the challenge weeks was started with the first challenge on day 26.

mine whether or not airway obstruction occurred during allergen challenge in sensitized and nonsensitized mice.

The results demonstrate that in our model AHR occurred first, but after several allergen challenges, an obstructive response during challenge can also be elicited.

Materials and Methods

Animals

Pathogen-free female BALB/c mice (BGVV, Berlin, Germany) aged 6–8 weeks weighing 18–22 g were held under standard conditions, fed an ovalbumin (OVA)-free diet and supplied with water ad libitum. The values of breathing parameters of spontaneously breathing BALB/c mice were determined under standard conditions at room air and room temperature. The experiments were carried out with the approval of the governmental authority (Landesamt für Arbeitsschutz, Gesundheitsschutz und Technische Sicherheit, Berlin).

Study Design

The study design is depicted in figure 1, which presents the various analyses and the groups of mice including the number per analysis. The following study groups were examined: (1) NIL, untreated

Body-Plethysmography

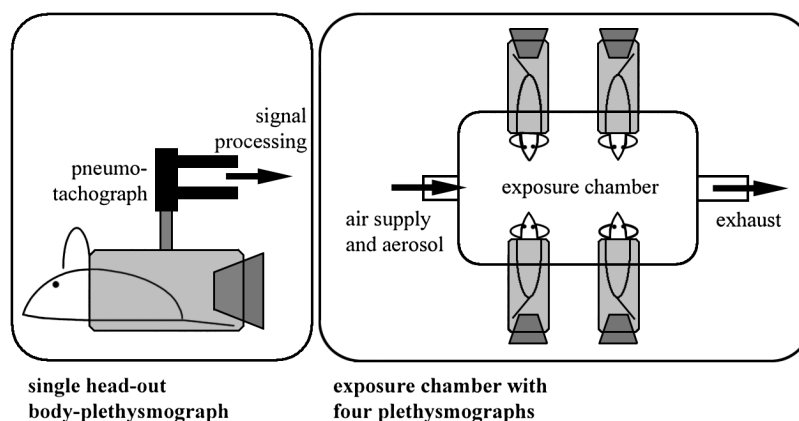


Fig. 2. Schematic drawing of the head-out plethysmograph system used for the measurement of breathing patterns in BALB/c mice. The apparatus was modified from [22]. See Material and Methods for further details.

control mice; (2) PBS/OVA, sham sensitization and OVA challenges; (3) OVA/PBS, OVA sensitization and sham challenges, and (4) OVA/OVA, OVA sensitization and OVA challenges.

In all groups, OVA-induced acute airway obstruction, AHR, airway resistance, serum immunoglobulin concentrations and cytokine levels in bronchoalveolar lavage (BAL) were assessed. Acute airway obstruction was measured during the first challenge of each week (days 26, 33, 40 and 47, respectively). Measurements of airway resistance with a tracheal cannula were carried out on day 28 as a validation experiment (4 groups, $n = 12$ each). The development of AHR was assessed by methacholine challenge 24 h after the second OVA challenge of each week (days 28, 35, 42 and 49, respectively). Serum immunoglobulin concentrations and cytokine levels in BAL were assessed on days 28 and 49.

Sensitization Protocol

Mice were sensitized to OVA by 3 intraperitoneal injections (10 μ g OVA grade VI, Sigma, Deisenhofen, Germany, adsorbed to 1.5 mg diluted in 200 μ l PBS) on days 1, 14 and 21 (fig. 1). The mice were challenged with OVA aerosol (1% in PBS) via the airways twice a week on consecutive days over a period of 4 weeks (1st–4th challenge week). Challenges were given on days 26 and 27 (1st week), 33 and 34 (2nd week), 40 and 41 (3rd week), and 47 and 48 (4th week). Sham sensitization and challenge were done with sterile Al(OH)₃-absorbed PBS.

Measurement of Serum Immunoglobulins

Total IgE and allergen-specific IgE, IgG₁, and IgG_{2a} antibody concentrations were measured by ELISA as previously described [18, 24]. Anti-mouse IgE, IgG₁ or IgG_{2a} monoclonal antibodies were obtained from Pharmingen (Hamburg, Germany).

Assessment of Leukocyte Distribution in BAL Fluids

Twenty-four hours after the last challenge animals were sacrificed, the trachea cannulated and BAL was performed by two lavages with 0.8 ml ice-cold PBS. The BAL fluid of each animal was pooled

and the recovered volume and the total cell number were determined. The mean recovery volume was 1.4 ± 0.2 ml, and no significant difference was detected between the study groups. Cytospins were prepared for each sample by centrifugation of 50 μ l BAL fluid ($100 \times g$, 5 min). After fixation, cytopins were stained with Diff Quik (Baxter Dade, Düringen, Switzerland). Cells were classified as either macrophages, neutrophils, eosinophils or lymphocytes, using standard morphologic criteria. Cell-free lavage fluids were stored at -20°C until analysis.

Measurement of Cytokines in Bronchoalveolar Fluids

The cytokines IL-4, IL-5 and IFN- γ were measured as previously described [18]. Antibodies were obtained from Pharmingen. Sensitivities were 10 pg/ml for IL-4, 30 pg/ml for IL-5, 10 pg/ml for IL-2 and 100 pg/ml for IFN- γ .

Exposure Apparatus

The exposure chamber (Crown Glass, Somerville, N.J., USA) consisted of a 2.5-liter glass cylinder with a baffle at the entrance (fig. 2) [22, 25]. Four body plethysmographs (Crown Glass), consisting of glass tubes, were attached to this chamber via ground glass joints. A neck collar was fixed at the front of each body plethysmograph. The collar consisted of dental latex rubber (Roeko, Langenau, Germany) with an 8-mm (ID) hole. An adhesive duct tape with a central opening (10 mm diameter) was mounted concentrically over the latex rubber for fixation.

Head-Out Body Plethysmography

Airflow was measured with a PTM 378/1.2 pneumotachograph (Hugo Sachs Electronics, March-Hugstetten, Germany) and a 8-T2 differential pressure transducer (Gaeltec, Dunvegan, UK). These devices were attached to the top port of each plethysmograph. The amplified airflow signal was visualized by an oscilloscope (Combi-graf 4; Gould, Dietzenbach, Germany) and digitized by an A/D converter (DAS-16; Keithley, Germering, Germany) for further computation.

Table 1. Respiratory parameters calculated from airflow signals

Parameter	Abbr.	Definition	Value	Unit
Tidal volume	VT	volume of air inhaled/exhaled per breath	0.146 ± 0.012	ml
Respiration frequency	F	number of breaths per minute	276 ± 23	bpm
Time of inspiration	TI	duration from minimum to maximum VT	0.069 ± 0.007	s
Time of expiration	TE	duration from maximum to minimum VT	0.137 ± 0.007	s
Mid-expiratory flow	EF ₅₀	airflow at 50% VT during expiration	-1.856 ± 0.239	ml/s
Baseline lung resistance	R _L	R _L determined according to Amdur and Mead [23]	0.209 ± 0.028	cm H ₂ O/ml/s

Approximately 4,000 breaths were measured for each mouse over a period of 15 min (means and standard deviation, n = 86). The baseline R_L values for mice fitted with a tracheal cannula are also given (n = 16). The baseline parameters were gained from pilot experiments with untreated normal (NIL) BALB/c mice.

Generation of Aerosols

Aerosols were generated with a common jet nebulizer (Pari-Boy®; Pari-Werke, Starnberg, Germany). The generator (Pari-Master®) produces a pressure of 1.4–1.5 bar and an airflow of 20 liters/min. The median mass diameter was 3.6 µm and the mass portion with a size below 5 µm was 65%. Methacholine was delivered with the Pari-Boy. Each concentration (0–30 mg/ml in PBS) was aerosolized for 5 min, with a recovery period of 5 min between administrations. Measurements were performed on the 3rd day of each challenge week.

Collection of Data

The computer programs used in this study were described previously [22, 25]. Briefly, data (digitized airflow signal described above) were collected over 14 s with a 1-second interval for computation of breaths per minute (F), tidal volume (VT), time of inspiration (TI), time of expiration (TE) and air flow during expiration at mid-tidal volume (EF₅₀). Each respiratory variable was calculated by the computer program exactly as previously described [25]. For these experiments, the mice were placed in the body plethysmographs and allowed to acclimatize for about 15 min. Then, the computer program calculated the above respiratory variables for each breath for a period of 15 min while the animals breathed room air. During this period, approximately 4,000 breaths per mouse were collected (since the number of breaths/minute is about 276) (table 1). The average values for each respiratory variable ($\pm$ SD) were then obtained. These values were used as the 'normal' or 'baseline' or 'control' values from which increases or decreases were calculated during the challenge period.

Determination of Airway Responsiveness

Mice (n = 8 per group) were challenged with OVA aerosol on the 1st and 2nd day of each week. Measurements of airway responsiveness were performed 24 h after the last challenge each week. Airway responsiveness was assessed by head-out body plethysmography as described previously [22]. Airflow signals were recorded in response to various concentrations of methacholine. Methacholine was aerosolized with a Pari-Jet nebulizer in increasing concentrations from 0 to 30 mg/ml in PBS. Each concentration was delivered for 5 min, with a recovery period of 5 min between administrations. The concentration of methacholine that caused a 50% reduction in expiratory airflow (MCh₅₀, mg/ml) was determined.

Measurements of Airway Resistance in Mice Fitted with a Tracheal Cannula

Mice were challenged with OVA aerosol on days 26 and 27. On day 28, 24 h after the last aerosol exposure, all animals (n = 12, each group) were prepared for assessment of airway responsiveness to methacholine as previously described [8]. Control mice (NIL) received PBS aerosol. The following groups were analyzed and compared to NIL: OVA/OVA, OVA/PBS, and PBS/OVA (see also schematic protocol, fig. 1). Animals were anesthetized intraperitoneally by an injection of pentobarbital (60 mg/kg body weight). A tracheal cannula was inserted via a midcervical incision, and a small polyethylene catheter was inserted into the jugular vein.

Spontaneous respiration was stopped by an intravenous injection of pancuronium bromide (1 mg/kg). The animals were ventilated with oxygen-enriched air, delivered by a Palmer respirator (VT 0.5 ml; respiratory rate 135 breaths/min; Bioscience, Sheerness, UK). As a parameter of airway caliber, lung resistance (R_L) was recorded and continuously computed (PMS 800; Mumed, London, UK) according to the principles described by Amdur and Mead [23]. Flow was measured over the tracheal cannula and transpulmonary pressure at points of equal volume during the respiratory cycle. Flow was measured with a Fleisch 0000 pneumotachograph (Fleisch, Lausanne, Switzerland), whereas transpulmonary pressure was measured with a differential pressure transducer, one end being connected to the outlet of the tracheal cannula, the other end connected to a needle inserted into the pleural space. Methacholine was administered intravenously with a microinfusion pump in increasing doses (0.16, 1.6, 4.8, 16, and 48 mg/kg) until at least a 40% increase in R_L was observed. The ratio between the changes at different concentrations was calculated.

Statistical Analysis

Results are presented as mean values and standard deviations unless otherwise stated. Student's t test was used to determine the level of significance.

For multiple comparisons of the results of weekly challenges with OVA aerosol in OVA-sensitized mice, the individual EF₅₀ values were entered into a worksheet (SigmaStat Version 2.0; Jandel, San Rafael, Calif., USA) for one-way analysis of variance (ANOVA), using p < 0.05 as the level of significance. Following this analysis, pairwise comparisons were performed using Dunnett's method to identify significant (p < 0.05) differences between the PBS/OVA group (used

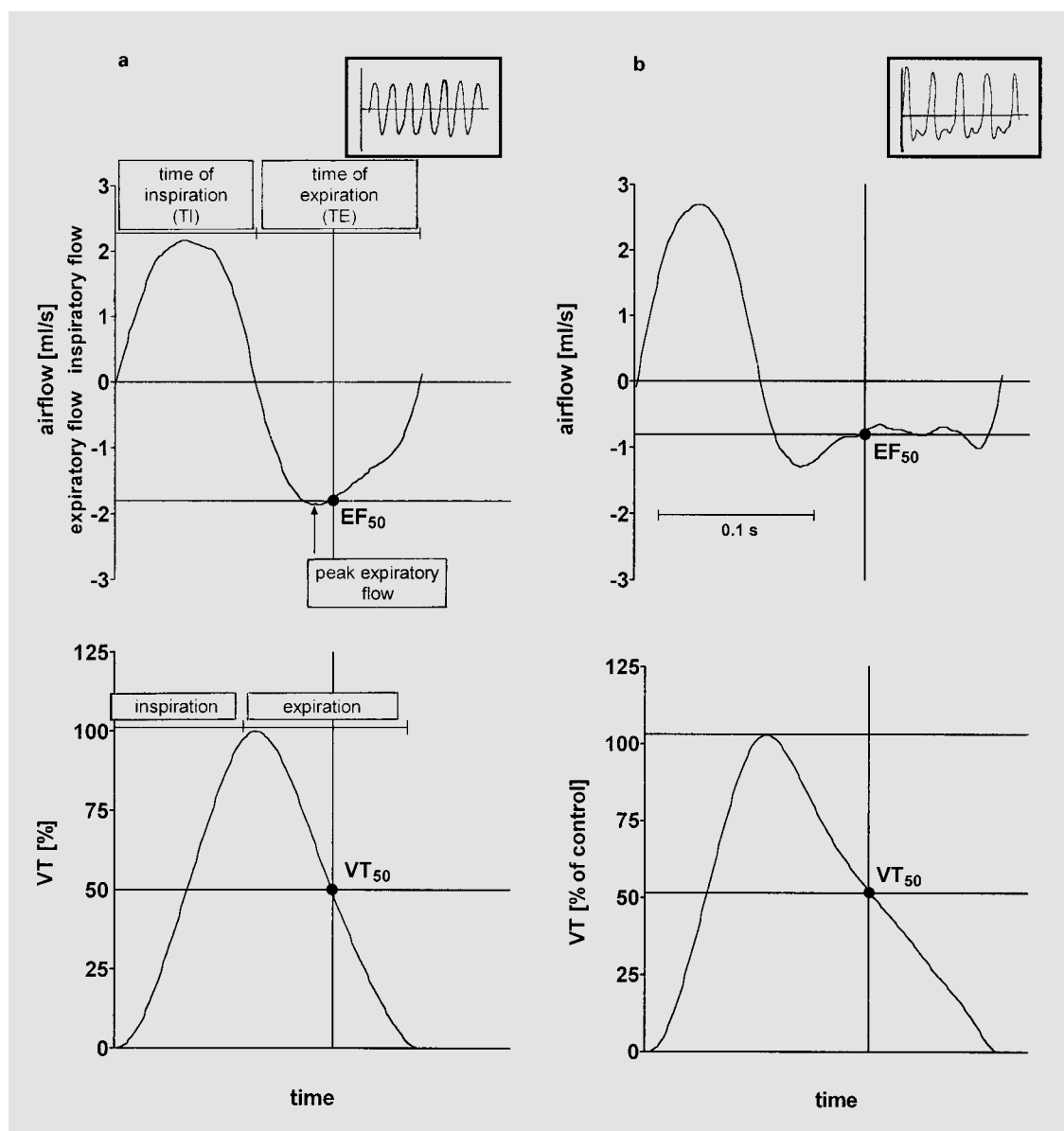


Fig. 3. Characteristic modifications to the normal breathing pattern in BALB/c mice (NIL). Inserts: several breaths. VT integrated by the computer program from the collected voltage digitizations. **a** Normal breathing pattern of BALB/c mice while breathing room air. **b** Characteristic pattern of airflow limitation, as assessed during exposure to methacholine (20 mg/ml), showing the decrease in EF₅₀ at VT₅₀.

as the control) and the OVA/OVA challenges. Following this first analysis, the same worksheet was used to conduct a one-way repeated measures ANOVA to compare the baseline EF₅₀ values obtained prior to challenge for each OVA/OVA group with the values obtained during challenge with OVA aerosol as well as after challenge. Following ANOVA, multiple comparisons were conducted using Dunnett's method, using $p < 0.05$ as the significance criterion.

Results

Antibody Production and Airway Inflammation

Mice were immunized according to Herz et al. [18] resulting in increased total IgE, OVA-specific IgE, IgG₁ and IgG_{2a} titers in the serum (data not shown). Upon allergen challenges with OVA, these mice (OVA/OVA) developed, in the 1st challenge week, airway inflamma-

Table 2. Antibody concentration in serum and cellular composition of BAL cells

	PBS/OVA 1st week	OVA/PBS 1st week	OVA/OVA 1st week	OVA/OVA 4th week
<i>Antibodies</i>				
Total IgE, ng/ml	31 ± 12	1,202 ± 508*	1,193 ± 396*	3,893 ± 406*
Anti-OVA IgE, LU/ml	16 ± 8	377 ± 613*	374 ± 438*	774 ± 479*
Anti-OVA IgG ₁ , LU/ml	44 ± 17	1,411 ± 538*	2,033 ± 1,052*	2,733 ± 1,231*
Anti-OVA IgG _{2a} , LU/ml	66 ± 29	562 ± 323*	808 ± 503*	798 ± 512*
<i>Cellular composition</i>				
Lymphocytes, × 10 ³	2.1 ± 1.6	1.6 ± 3.3	90 ± 21*	100 ± 41*
Neutrophils, × 10 ³	2.6 ± 1.9	1.6 ± 1.3	25 ± 46*	78 ± 43*
Eosinophils, × 10 ³	<1	<1	80 ± 19*	493 ± 212*
Monocytes, × 10 ³	226 ± 105	205 ± 98	102 ± 25*	99 ± 41*

Means and standard deviation (n = 10 mice of each group). * p < 0.05, Student's t test values significantly different compared to PBS/OVA mice.

tion with lung eosinophilia (table 1) accompanied by an increase in TH2 cytokines IL-4 and IL-5 in the BAL [Braun et al., in preparation]. Additional challenges further increased these parameters, with the exception of IL-4 which decreased to baseline levels. IFN- γ remained basically unchanged over the entire study period. Airway inflammation was not present in PBS/OVA or OVA/PBS control groups.

Breathing Pattern of BALB/c Mice

Head-out body plethysmography [22, 25, 26] was used to assess changes in the respiratory pattern of sensitized mice. EF₅₀ during normal breathing was calculated from digital recordings for each breath.

Recordings of spontaneous breathing revealed a sinusoidal pattern of the airflow in NIL mice (fig. 3a, top and insert). Positive values indicate inspiration, whereas negative values indicate expiration. VT was calculated from the airflow by integration (fig. 3a bottom). EF₅₀ is the expiratory airflow when 50% of the tidal volume (VT₅₀) is exhaled, previously abbreviated VD [22]. Baseline values of these respiratory variables measured in BALB/c mice are listed in table 2.

In a first set of experiments, the airway response to methacholine was determined in NIL mice which did not differ from sham-sensitized (OVA/PBS) or sham-challenged mice (fig. 4a,b). Methacholine (15 mg/ml in PBS) significantly changed the normal breathing pattern in mice to a characteristic appearance typically observed after administration of bronchoconstrictors [22, 27, 28] as shown in figure 3b. TE was prolonged by about 200%, whereas the breathing rate and EF₅₀ were decreased by

about 50%. Peak expiratory flow was also halved. The reduction in the ratio of the time needed to reach maximal tidal expiratory flow (TME) divided by total TE has been previously demonstrated to indicate bronchoconstriction [27]. In our experiment, methacholine reduced TME/TE from 37 to 11%. TI and peak inspiratory flow, in contrast, remained basically unchanged compared to normal.

AHR in Response to Methacholine

We then used head-out body plethysmography to examine the differences in the response to methacholine of mice sensitized to OVA which were either challenged twice with OVA aerosol (OVA/OVA) or challenged with PBS aerosol (OVA/PBS). These mice were exposed to increasing methacholine concentrations. Each concentration was applied for 5 min. EF₅₀ values displayed a dose-response relationship, with a significant shift to the left for the OVA/OVA group in comparison to the PBS/OVA group (fig. 4b). The concentration of methacholine that caused a decline of 50% in EF₅₀ (MCh₅₀) was significantly lower in OVA/OVA mice (MCh₅₀: 5.7 ± 2 mg/ml methacholine) compared to control groups (PBS/OVA, MCh₅₀: 15 ± 2 mg/ml; OVA/PBS, MCh₅₀: 15.5 ± 2 mg/ml). The dose-response for NIL mice did not differ from PBS/OVA or OVA/PBS mice and the MCh₅₀ was comparable (15.1 ± 1.9 mg/ml). These measurements were done in the 1st week. The AHR persisted, but did not increase further during the following weeks when using the same challenging methacholine protocol (data not shown).

In order to confirm these findings, measurements of airway resistance were carried out in mice fitted with a

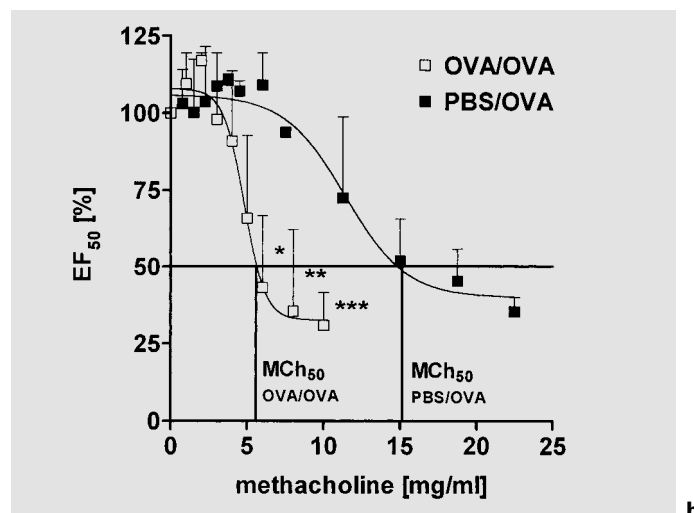
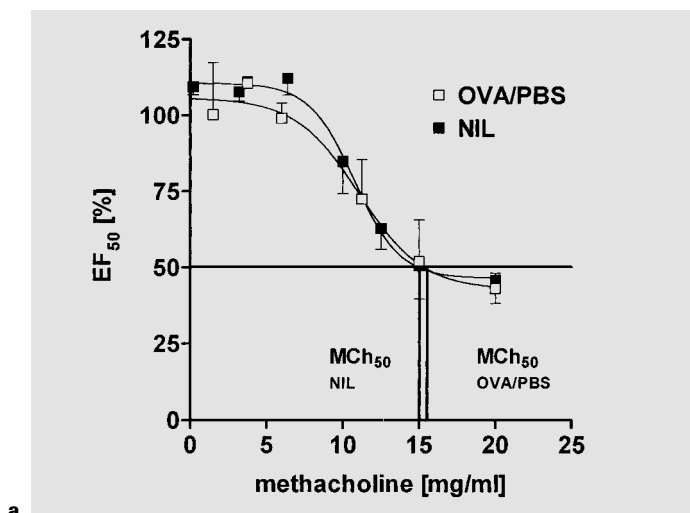
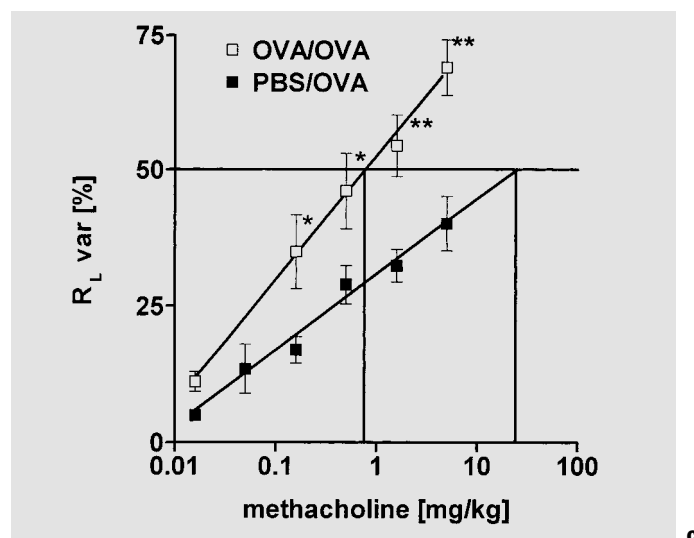


Fig. 4. Assessment of airway obstruction in BALB/c mice in the 1st challenge week 24 h after the last aerosol challenge with two different methods. Mice were sensitized and challenged according to the protocol. **a, b** Assessment of AHR by head-out body plethysmography. The error bars represent standard error of mean (SEM; $n = 8$ animals in each group). Student's t test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. **c** Assessment of AHR by measurement of airway resistance (R_L) in mice fitted with a tracheal cannula. Ordinate = variation of R_L as percentage of control. $n = 12$ in each group.



tracheal cannula on day 28, 24 h after the last OVA challenge. Intravenous methacholine challenge (range 0–4.8 mg/kg) revealed a significantly higher increase in resistance in OVA/OVA mice compared to OVA/PBS mice (fig. 4b).

Measurement of Acute Airway Obstruction during OVA Challenge

In order to measure the possible acute obstructive effects during OVA challenges, OVA-sensitized mice were repeatedly exposed to OVA aerosol in the exposure apparatus twice weekly over a period of 4 weeks. Each challenge lasted 20 min. Continuous measurements of EF_{50} were obtained during challenge and for a period of 40 min after challenge.

During the first challenge with OVA, no change was observed in the breathing pattern in neither OVA/OVA nor PBS/OVA mice, and the EF_{50} values remained within the control values (fig. 5a,b). In the following weeks, changes in the OVA/OVA mice breathing pattern were observed but not in the PBS/OVA mice. In the 2nd week, the baseline values of EF_{50} of OVA/OVA mice were already reduced prior to the challenge, probably due to the airway inflammation that developed since the last challenge. This effect was more pronounced over the following weeks as the number of OVA challenges increased. The lowest EF_{50} baseline values were reached after the 7th challenge in the 4th week (1.22 ml/s, 68% of control) (fig. 5b). An acute-type obstructive response upon OVA challenge was first observed in the 2nd week and was

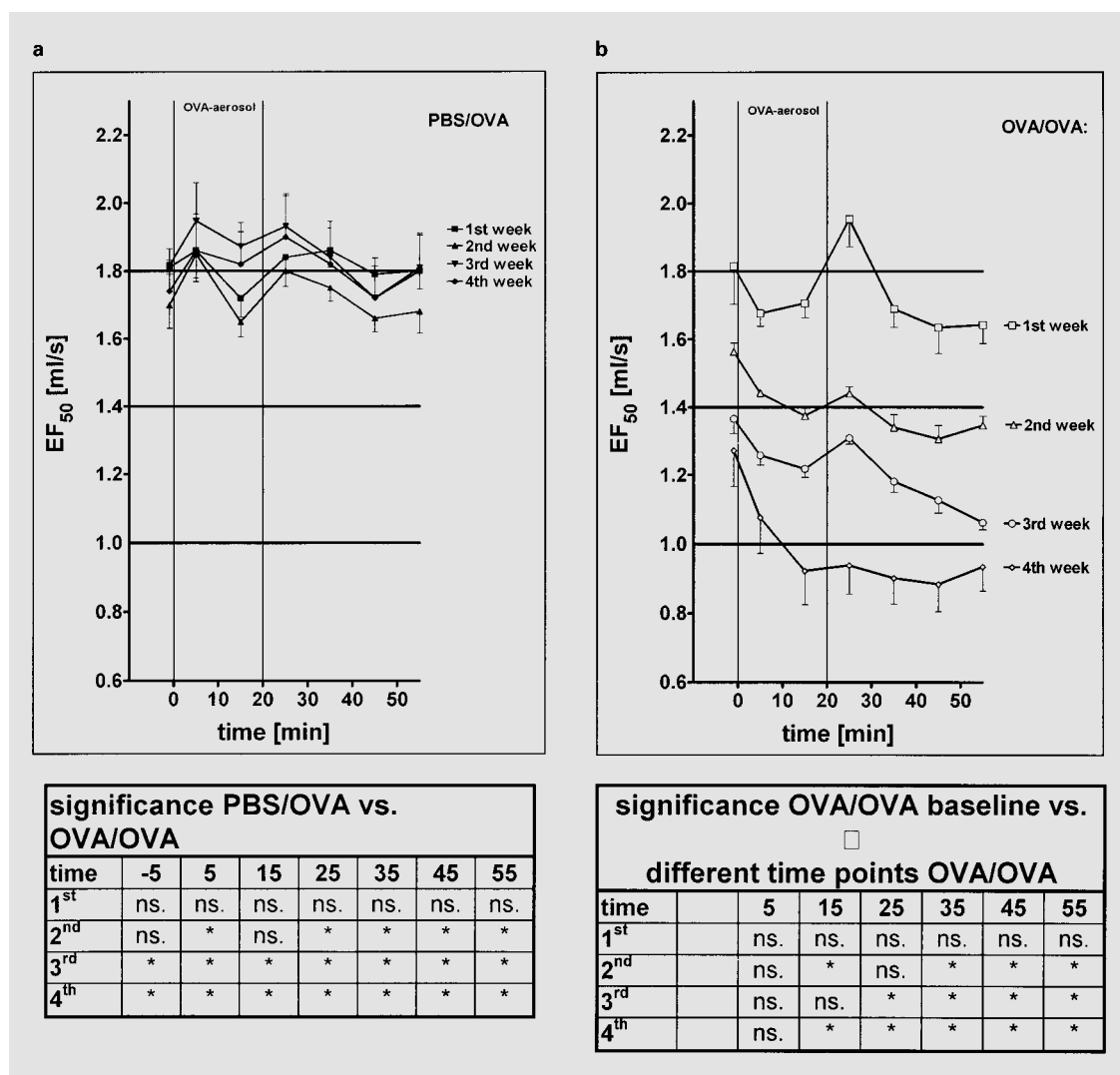


Fig. 5. Airway obstruction in OVA/OVA mice. Measurement of EF_{50} in response to different repetitions of OVA challenges: see figure 1. OVA aerosol was administered from 0–20 min. **a** PBS/OVA: sham sensitization and OVA challenge. **b** OVA/OVA: OVA sensitization and challenge. The error bars represent standard errors of the mean. Student's t test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $n = 8$.

more pronounced during the following weeks. The response appeared almost immediately after the beginning of the challenge as indicated by rapidly decreasing EF_{50} values during the first 10–15 min of challenge. Afterwards, EF_{50} values declined more slowly reaching minimal levels 45 min after onset of the challenge. The maximum decrease in EF_{50} was measured in the 4th week (0.8 ml/s, 44% of control). No spontaneous recovery occurred during the observation period, but 24 h later the EF_{50} values returned to the baseline values measured before this challenge (data not shown).

The analysis of single breaths in the 4th week revealed that prior to OVA challenge the baseline values of EF_{50} and VT were reduced, but no major changes in the pattern were detected (fig. 6). In contrast, after OVA challenge (55 min) obvious changes occurred. EF_{50} and peak flow were reduced and TE was prolonged.

In order to confirm the allergen-specific nature of the airway obstruction, a BSA aerosol was given to the OVA/OVA mice in the 4th week, resulting in a minor insignificant decrease in EF_{50} (data not shown).

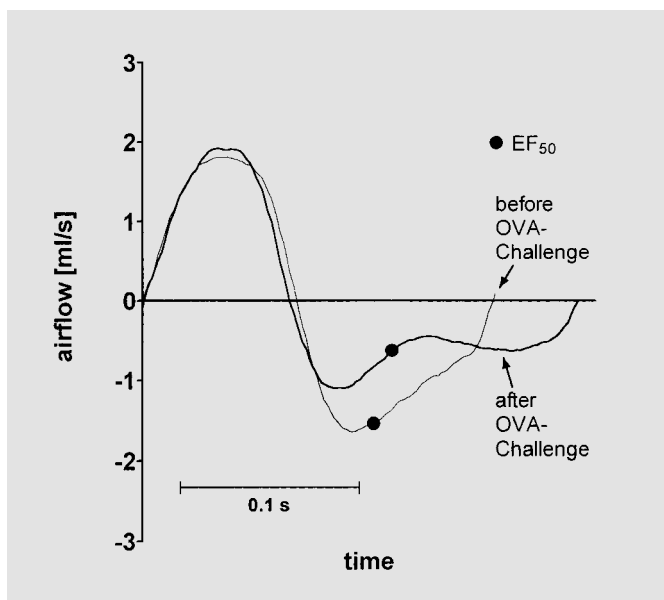


Fig. 6. Breathing pattern in OVA-sensitized mice in the 4th week. The airflow tracings shown are for the same animals prior to and following 55 min of OVA challenge. EF_{50} values are shown as calculated by the computer program.

Discussion

A well-characterized model of allergic inflammation in mice [18] was used to study both AHR and a possible acute-type airway reaction during allergen challenge. AHR has already been demonstrated in comparable models by using electric field stimulation of tracheal smooth muscle [18], by measurement of airway resistance obtained in tracheally cannulated mice with intravenous acetylcholine challenge [8], and in mice challenged with an aerosol of methacholine followed by measurement of an index of breathing difficulty during expiration using whole-body plethysmography [19]. The development of AHR has been attributed to the inflammatory response in OVA/OVA mice which is characterized by a cellular influx consisting predominantly of lymphocytes and eosinophils associated with increased levels of the TH2 cytokines IL-4 and IL-5 [18].

Despite the fact that AHR has been demonstrated, an acute airway response during allergen challenge has not yet been demonstrated in mice sensitized according to similar protocols. In this study, we also demonstrated the onset of AHR by using a different measurement, EF_{50} , during challenge with methacholine. This AHR finding was confirmed by using a second method that measures

airway resistance in mice fitted with a tracheal cannula according to the classical principles of Amdur and Mead, as previously described [8]. AHR was observed in sensitized mice on day 28, 24 h after the last OVA aerosol challenge. Secondly, we showed a decrease in EF_{50} during challenge with OVA. Thus an acute airway reaction was obtained in this model. It is an important finding that the development of AHR preceded the development of acute airway obstruction in response to allergen challenges. This sequential occurrence of airway obstruction after AHR onset may be the reason why positive responses to allergen provocation tests in mice have not yet been reported in the literature. In addition, there are several reports, particularly in children, demonstrating that AHR precedes the development of acute asthmatic responses [2, 29, 30].

Upon OVA challenge, a specific decrease in EF_{50} was noted, indicating that airway obstruction, as observed with methacholine challenge, was occurring. This effect increased from week to week. The kinetics of this response are compatible with an immediate-type allergic reaction. The rapid decrease in EF_{50} values with the lowest level reached after about 45 min resembles an IgE-dependent immediate-type allergic response. In humans, obstructive responses occur earlier than reported here in mice. Species differences, the level of sensitization and the concentration of allergen might contribute to different kinetics. For a concordant experimental design, a 20-min exposure time was chosen throughout the whole experiment. Five minutes of exposure, however, was sufficient to induce the described effects in the 4th week (data not shown). The response observed could only be elicited in allergen-sensitized mice which were challenged with the allergen. In contrast, PBS/OVA mice did not respond to OVA challenge with airway obstruction. In our model, allergen-induced acute airway obstruction was associated with an allergic immune response. Therefore this model can be used to delineate the cellular and molecular mechanisms that contribute to bronchial asthma, using EF_{50} as an indicator to select appropriate times to study the various elements involved. Candidates include IgE, mast cell, eosinophil and T-cell dependencies.

We also observed a decline in baseline EF_{50} values as mice were repeatedly challenged with OVA, indicating that the function of the airways was progressively impaired. This decline was most probably due to an aggravating pathological process. Airway narrowing, increased mucus production, inflammatory edema, neuronal alterations [Braun et al., in preparation] and possibly a change in muscle tone may be considered [29] to contrib-

ute to this process of declining EF₅₀ values with repeated challenges. Further studies will be required to analyze the underlying pathological mechanisms in detail.

Though the model presented here offers a good tool to study obstructive responses, there are differences to human asthma. First, the eosinophilia observed in the 4th week of exposure exceeds the increases reported in asthmatics after allergen challenge. However, the eosinophilia was determined only by BAL analysis, which may have only a limited relationship with the actual histologic appearance. Furthermore, a late-phase response was not detected. Finally, wheezing, coughing or fatal asthma attacks were not observed.

The method used here to measure EF₅₀ has previously been validated to indicate airway obstruction during aerosol challenge of mice and guinea pigs [22, 25]. Airway obstruction limits the expiratory airflow in mice, and a decrease in EF₅₀ was correlated with an increase in airflow resistance when simultaneously measured by the classic method introduced by Amdur and Mead [23]. As a result of airflow limitation, the expiratory flow pattern changed

from a sinusoidal to a quadrilateral shape (fig. 6) as typically observed during bronchoconstriction in humans as well [26, 27]. Thus, the method is well-suited not only to assess AHR during methacholine challenge but also to demonstrate the induction of an acute airway obstruction during allergen challenge. Most importantly, it permitted elucidation of a decrease in airway function with repeated challenges.

In summary, an acute airway obstruction was demonstrated during aerosol allergen challenge in mice. In our model, this response was observed after the development of an increased reactivity to methacholine. The degree of the acute response increased with repeated exposures and such exposures also resulted in diminished airway function as measured by EF₅₀ prior to each challenge.

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