

Martin Witzenrath, Birgit Ahrens, Stefanie M. Kube, Armin Braun, Heinz G. Hoymann, Andreas C. Hocke, Simone Rosseau, Norbert Suttorp, Eckard Hamelmann and Hartwig Schütte

Am J Physiol Lung Cell Mol Physiol 291:466-472, 2006. First published Apr 14, 2006;
doi:10.1152/ajplung.00011.2005

You might find this additional information useful...

This article cites 33 articles, 18 of which you can access free at:

<http://ajplung.physiology.org/cgi/content/full/291/3/L466#BIBL>

This article has been cited by 1 other HighWire hosted article:

Functional characterization of voltage-gated K⁺ channels in mouse pulmonary artery smooth muscle cells

E. A. Ko, E. D. Burg, O. Platoshyn, J. Msefya, A. L. Firth and J. X.-J. Yuan

Am J Physiol Cell Physiol, September 1, 2007; 293 (3): C928-C937.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Updated information and services including high-resolution figures, can be found at:

<http://ajplung.physiology.org/cgi/content/full/291/3/L466>

Additional material and information about *AJP - Lung Cellular and Molecular Physiology* can be found at:

<http://www.the-aps.org/publications/ajplung>

This information is current as of September 28, 2009 .

Detection of allergen-induced airway hyperresponsiveness in isolated mouse lungs

Martin Witzernath,^{1*} Birgit Ahrens,^{2*} Stefanie M. Kube,¹ Armin Braun,³ Heinz G. Hoymann,³ Andreas C. Hocke,¹ Simone Rosseau,¹ Norbert Suttrop,¹ Eckard Hamelmann,² and Hartwig Schütte¹

¹Department of Internal Medicine/Infectious Diseases and ²Department of Pediatric Pneumology and Immunology, Charité, University Medicine Berlin, Berlin; and ³Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany

Submitted 7 January 2005; accepted in final form 13 March 2006

Witzernath, Martin, Birgit Ahrens, Stefanie M. Kube, Armin Braun, Heinz G. Hoymann, Andreas C. Hocke, Simone Rosseau, Norbert Suttrop, Eckard Hamelmann, and Hartwig Schütte. Detection of allergen-induced airway hyperresponsiveness in isolated mouse lungs. *Am J Physiol Lung Cell Mol Physiol* 291: L466–L472, 2006. First published April 14, 2006; doi:10.1152/ajplung.00011.2005.—Airway hyperresponsiveness (AHR) is a hallmark of bronchial asthma. Important features of this exaggerated response to bronchoconstrictive stimuli have mostly been investigated in vivo in intact animals or in vitro in isolated tracheal or bronchial tissues. Both approaches have important advantages but also certain limitations. Therefore, the aim of our study was to develop an ex vivo model of isolated lungs from sensitized mice for the investigation of airway responsiveness (AR). BALB/c mice were sensitized by intraperitoneal ovalbumin (Ova) and subsequently challenged by Ova inhalation. In vivo AR was measured in unrestrained animals by whole body plethysmography after stimulation with aerosolized methacholine (MCh) with determination of enhanced pause (P_{enh}). Twenty-four hours after each P_{enh} measurement, airway resistance was continuously registered in isolated, perfused, and ventilated lungs on stimulation with inhaled or intravascular MCh or nebulized Ova. In a subset of experiments, in vivo AR was additionally measured in orotracheally intubated, spontaneously breathing mice 24 h after P_{enh} measurement, and lungs were isolated further 24 h later. Isolated lungs of allergen-sensitized and -challenged mice showed increased AR after MCh inhalation or infusion as well as after specific provocation with aerosolized allergen. AR was increased on days 2 and 5 after Ova challenge and had returned to baseline on day 9. AHR in isolated lungs after aerosolized or intravascular MCh strongly correlated with in vivo AR. Pretreatment of isolated lungs with the β_2 -agonist fenoterol diminished AR. In conclusion, this model provides new opportunities to investigate mechanisms of AHR as well as pharmacological interventions on an intact organ level.

airway inflammation; sensitized mice; airway resistance; ovalbumin; asthma

AIRWAY HYPERRESPONSIVENESS (AHR) is one of the cardinal features of bronchial asthma (21). AHR can be defined as an exaggerated response of the airways to a variety of nonspecific stimuli, resulting in airway obstruction (23, 25). However, the mechanisms contributing to this phenomenon are not exactly known. Several animal models and different measurement techniques have been used for the investigation of AHR (33). In recent years, new opportunities to study AHR pathophysiology essentially derived from murine models of allergic lung

inflammation (7, 8). Airway responsiveness (AR) in these mice was determined by both in vitro and in vivo techniques. In vitro, AR of immunized mice was measured on the level of tracheal smooth muscle contractility (18); in vivo, invasive techniques in anesthetized animals (20, 26) as well as noninvasive measurements using either restrained (22) or unrestrained (14) conscious mice were implemented. Beyond the analysis of smooth muscle activity, in vivo techniques may also reflect the impact of mucus production, mucosal edema, or other changes in the upper and/or lower airways. However, the interaction of pulmonary and systemic responses may complicate the interpretation of lung function and bronchial reactivity measurements in intact animals. Furthermore, pharmacological investigations are limited by systemic side effects and pharmacokinetic issues.

The isolated, blood-free perfused, and ventilated mouse lung (IPML) provides an intact organ model with functional integrity, which is independent from any metabolic and humoral impact of other organs (16, 17, 29). With this model, lung function parameters, e.g., airway resistance and dynamic compliance, are accessible to accurate measurement. Furthermore, the impact of pharmacological compounds that may be administered either intravascularly or via the airways by instillation or inhalation can easily be detected. In the present study, we analyzed for the first time AR in isolated lungs of allergen-sensitized and -challenged mice. We investigated the effects of ex vivo allergen provocation as well as inhalative and intravascular methacholine (MCh) challenge and compared AR of intact mice with AR of their respective isolated lungs. Furthermore, we investigated the time course of AHR after allergen sensitization and airway challenges, as well as the sensitivity of AHR to bronchodilators in the IPML. The basic features of AHR were preserved in IPML, thus allowing a more analytical approach at the intact organ level to delineate mechanisms of AHR.

METHODS

Ovalbumin sensitization and challenge. Animal studies were approved by governmental authorities. Pathogen-free female BALB/c mice (20–23 g; BgVV, Berlin, Germany) were maintained on an ovalbumin (Ova)-free diet. Systemic sensitization with Ova (20 μ g/injection; Sigma, Deisenhofen, Germany) adsorbed to 2 mg of Al(OH)₃ (Pierce, Rockford, IL) by intraperitoneal injections on days 0 and 14 and repeated airway challenges with aerosolized 1% (wt/vol) Ova in PBS (20 min) on days 28, 29, and 30 were performed as described previously (11).

* M. Witzernath and B. Ahrens contributed equally to this work.

Address for reprint requests and other correspondence: H. Schütte, Charité, Dept. of Internal Medicine/Infectious Diseases, Univ. Medicine Berlin, Augustenburgerplatz 1, 13353 Berlin, Germany (e-mail: hartwig.schuette@charite.de).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

AR in unrestrained mice. AR was measured in unrestrained animals by barometric whole body plethysmography (WBP) (Buxco, EMKA Technologies, Paris, France) as described previously (14). Briefly, each mouse was placed in a main chamber of the body plethysmograph. Inhalative airway provocation was performed with aerosolized PBS (baseline) and increasing doses of MCh (6.25–50 mg/ml; Fährhaus, Hamburg, Germany) for 3 min each. Pressure differences between the main chamber and a reference chamber caused by volume and resultant pressure changes during inspiration and expiration of the mouse were measured and averaged for 3 min after each nebulization. As an index of in vivo airway obstruction, enhanced pause (P_{enh}) values were calculated (14). For correlation with ex vivo measurements, P_{enh} values detected after provocation with 25 mg/ml MCh were considered.

AR in intubated mice. AR was assessed in orotracheally intubated, spontaneously breathing mice as described previously (12). Briefly, mice were anesthetized and intubated under direct visualization. The animals were then placed in a supine position, and the cannulas were directly attached to a pneumotachograph (HSE-Harvard Apparatus, March-Hugstetten, Germany) connected to a differential pressure transducer (Validyne DP 45–14; HSE-Harvard) for monitoring of tidal flow. To measure transpulmonary pressure (P_{tp}), water-filled PE-90 tubing was inserted into the esophagus to the level of the midthorax and coupled to a pressure transducer (P75; HSE-Harvard). Airway resistance was determined from the ratio of P_{tp} to tidal flow over an entire breath cycle. After baseline values were recorded, AR to aerosolized MCh in increasing doses (0.0625, 0.125, 0.25, 0.5, and 1.00 μg) was assessed. Dried aerosols were generated by a computer-controlled, jet-driven aerosol generator system (Bronchy III, Fraunhofer ITEM; licensed by Buxco, Troy, NY). Aerosol concentrations were determined by a gravimetrically calibrated photometer. The total MCh dose inhaled was controlled by a computerized dose-control system based on the continuously measured respiratory volume per minute and aerosol concentration.

Isolated, perfused mouse lung. Mouse lungs were prepared, ventilated, and perfused as described previously (16, 17, 29, 31). Briefly, anesthetized mice were tracheotomized and ventilated. After sternotomy and cannulation of left atrium and pulmonary artery, heart and lungs remained in the open chest, lungs were perfused with 37°C sterile Krebs-Henseleit-hydroxyethylamylpectin buffer (1 ml/min; Serag, Germany) in a nonrecirculating system, and left atrial pressure was adjusted to +2.2 cmH₂O. Pulmonary arterial and left atrial pressures were monitored continuously. On isolation, lungs were ventilated by negative pressure (–4.5 to –9.0 cmH₂O, 90 breaths/min) in a closed chamber. Hyperinflation (–24 cmH₂O) was performed at 4-min intervals. The chamber pressure was continuously measured by a differential pressure transducer, and airflow velocity was monitored by means of a pneumotachograph connected to a second differential pressure transducer. All data were amplified and analyzed with Pulmodyn software, and the data for airway resistance were analyzed as described previously (17). All hardware and software were purchased from HSE-Harvard Apparatus. Aerosol was generated with a Pari LL nebulizer (Pari, Starnberg, Germany) connected to a custom inhalation device.

Ex vivo AR in IPML. After a steady-state period of 30 min, MCh was nebulized for 1 min or administered to the perfusate for 30 s at 12-min intervals. In a separate set of experiments addressing the impact of specific provocation, Ova was nebulized for 20 min, and airway resistance was measured over a period of 60 min. Airway resistance values were determined at the end of the steady-state period as well as at the maximum level of resistance increase. The change in airway resistance was expressed as fold airway resistance.

Persistence of AHR. P_{enh} measurements were performed in allergen-sensitized mice at 1, 4, 8, or 13 days after completion of the Ova sensitization and challenge protocol. Twenty-four hours after the P_{enh} measurement, the rise of airway resistance in response to intravascular 10 μM MCh was measured in the isolated organ.

Impact of fenoterol on airway responses in IPML. Lungs of allergen-sensitized mice were ventilated and perfused ex vivo, and 10 μM MCh was added to the perfusate for 30 s repetitively at 12-min intervals. Nine minutes before each MCh administration, increasing doses of the β_2 -agonist fenoterol were continuously added to the buffer fluid. AR was determined as described above.

Data analysis. Data are expressed as means $\pm$ SE. Differences were analyzed by one-way ANOVA, followed by post hoc Student-Newman-Keuls test. Correlations were expressed by Spearman's correlation coefficient, with P values being two tailed. P values of <0.05 were considered to represent a significant difference or correlation. All calculations were performed with GraphPad 4 software (San Diego, CA).

RESULTS

AHR in IPML in response to aerosolized MCh. In a first set of experiments, BALB/c mice were sensitized and challenged to Ova or sham treatment as described above (Ova-Ova, Ova-PBS, or PBS-PBS, respectively). AR was measured on day 31 in vivo by WBP and on the consecutive day (day 32) in the IPML, each in response to inhaled MCh. Baseline resistance and dynamic compliance values in IPML did not differ significantly among the experimental groups (resistance: Ova-Ova 1.51 ± 0.06 , Ova-PBS 1.42 ± 0.09 , PBS-PBS 1.41 ± 0.11 cmH₂O $\cdot$ s $\cdot$ ml^{–1}; compliance: Ova-Ova 37.10 ± 2.05 , Ova-PBS 39.74 ± 3.83 , PBS-PBS 40.19 ± 2.76 $\mu\text{l}/\text{cmH}_2\text{O}$), and nebulized NaCl (0.9%) did not cause any significant change in airway resistance or dynamic compliance in any experimental group (Fig. 1, A and B). Isolated lungs of allergen-sensitized and -challenged mice showed a pronounced dose-dependent increase in airway resistance to inhalative MCh provocation, peaking ~ 1.5 min after onset of nebulization and returning to prenebulization values after another ~ 4 min (6.25 and 12.5 mg/ml MCh) or ~ 10 min (25 mg/ml MCh). Dynamic compliance decreased distinctly in lungs of Ova-Ova mice. In contrast, lungs of Ova-PBS and PBS-PBS mice responded with significantly lower changes in airway resistance and dynamic compliance, respectively (Fig. 1, A and B). Inhalative MCh challenge did not affect pulmonary arterial pressure in any experimental group (data not given in detail).

AR to inhaled MCh in IPML: correlation to AR in intact mice. AR in intact mice was compared with AR to inhaled MCh in their respective isolated lungs. In vivo measured average fold increases of P_{enh} and ex vivo determined fold airway resistance (at 25 mg/ml MCh; Fig. 1C) showed significant correlation. In a separate set of experiments, in vivo AR of unrestrained mice was measured on day 31 of the experimental protocol, and 24 h later the same mice were anesthetized and orotracheally intubated. AR to inhaled MCh (total inhalative dose 0.25 μg) was measured in spontaneously breathing animals. A further 24 h later, the lungs of these animals were isolated and ex vivo AR to MCh aerosol (25 mg/ml) was measured. Each AR measurement showed significant correlation with both other measurements (Fig. 2).

Inhalative provocation with Ova in IPML. Mice were Ova or sham sensitized and challenged. On day 32, lungs were isolated and exposed to 5% Ova aerosolized for 20 min, and early-phase airway responses were continuously monitored for a period of 60 min. A moderate, but significantly higher, airway response was noted in lungs of Ova-Ova mice compared with Ova-PBS and PBS-PBS mice (Fig. 3).

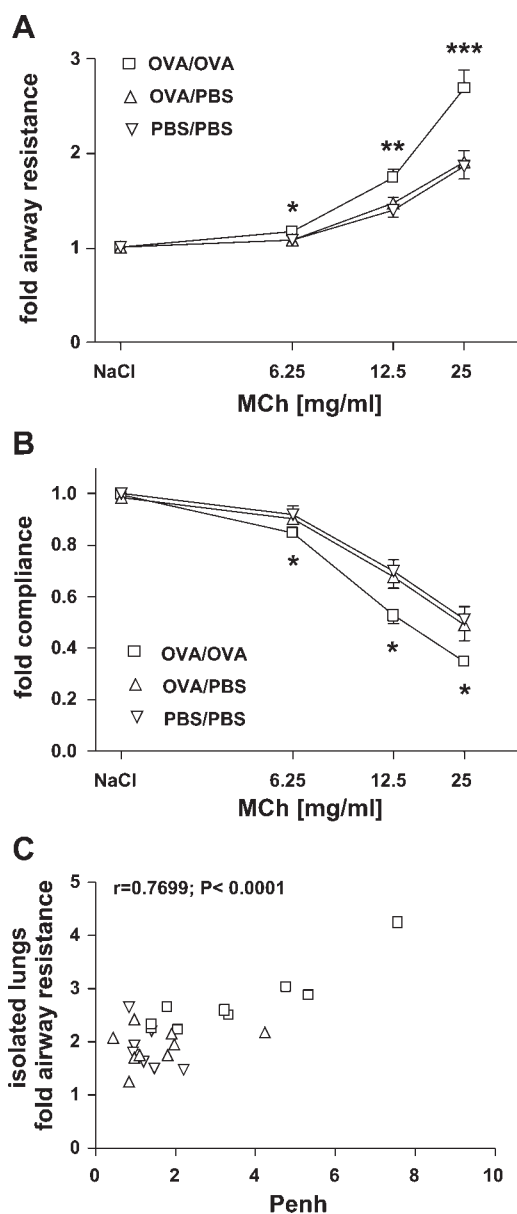


Fig. 1. Airway responsiveness (AR) to inhalative methacholine (MCh) provocation in isolated mouse lungs. Mice were sensitized to ovalbumin (Ova) adsorbed to $\text{Al}(\text{OH})_3$ by intraperitoneal injections or received vehicle on days 0 and 14. On days 28, 29, and 30, airway challenges were performed with 1% (wt/vol) Ova diluted in PBS or with PBS alone delivered by 20-min aerosolization. In the ex vivo perfused and ventilated lung (day 32 of the protocol), 0.9% NaCl and increasing doses (6.25, 12.5, 25 mg/ml) of MCh were nebulized for 1 min each. Airway resistance (A) and dynamic compliance (B) were measured continuously, and changes were expressed as fold airway resistance or fold dynamic compliance, respectively; $n = 8$ each. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Ova-PBS and PBS-PBS. Values are means $\pm$ SE. Error bars are not shown when symbols are larger than bars. C: correlation between airway responsiveness (AR) in unrestrained mice and AR in isolated mouse lungs, both determined by inhalative MCh provocation. AR was measured in unrestrained, conscious mice on day 31. MCh (25 mg/ml) was nebulized for 3 min each, and enhanced pause (P_{enh}) values were determined. Subsequently, AR was measured in isolated, perfused lungs 24 h later, considering baseline values before nebulization of 25 mg/ml MCh and at maximum airway resistance. Data were analyzed with Spearman's correlation test (r = correlation coefficient); P values are 2 tailed.

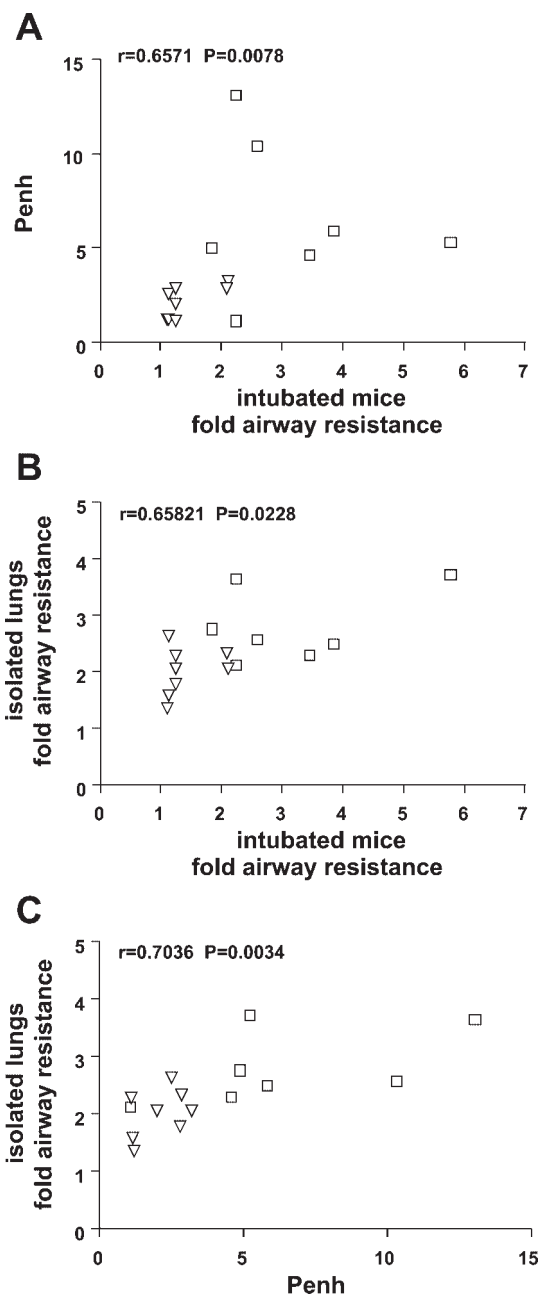


Fig. 2. Correlation between AR in unrestrained mice, AR in intubated mice, and AR in isolated mouse lungs, each determined by inhalative MCh provocation. Mice were allergen sensitized and challenged according to the protocol. On day 31, AR was measured in unrestrained, conscious mice. MCh (25 mg/ml) was nebulized for 3 min each, and P_{enh} values were determined. Subsequently, AR to inhaled MCh (0.25 μg) was measured on day 32 in orotracheally intubated, spontaneously breathing mice. On day 33, AR to inhaled MCh (25 mg/ml) was measured in isolated, perfused lungs. Data were analyzed with Spearman's correlation test; P values are 2 tailed. □, Ova-Ova; ▽, PBS-PBS.

Suitability of intravascular MCh for ex vivo AHR detection in IPML. Perfusion of isolated Ova-Ova, Ova-PBS, and PBS-PBS lungs was changed every 12 min to buffer containing MCh (1, 3, 10 μM) for 30 s each, and the airway responses were monitored (Fig. 4A). On day 32, isolated lungs of allergen-sensitized and -challenged mice showed significantly enhanced airway responses compared with Ova-PBS and PBS-

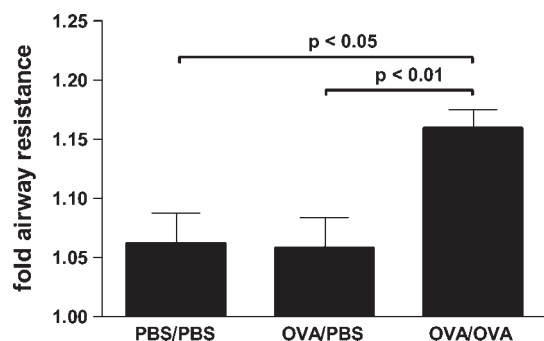


Fig. 3. AR to inhalative Ova provocation in isolated mouse lungs. Mice were allergen sensitized and challenged. On day 32, in the ex vivo perfused and ventilated lung Ova (5%) was nebulized for 20 min. AR was determined considering values 30 s before Ova nebulization and at maximum airway resistance. Values are means $\pm$ SE; $n = 6$.

PBS lungs (Fig. 4, B and C). Pulmonary arterial pressure was not affected by intravascular MCh (Fig. 4A).

Time course of AHR. To delineate the temporal kinetics of allergen-induced AHR, we investigated mice and their isolated lungs at additional time points. Four, eight, or thirteen days after termination of the sensitization and challenge procedure at day 30 (i.e., day 34, 38, or 43 of the protocol), we first monitored P_{enh} in intact animals after inhalative MCh provocation and then determined AR after intravascular MCh provocation (10 μM) in isolated lungs of the same animals on the consecutive day (Fig. 5A). On day 35, the level of AHR detected in isolated lungs of allergen-sensitized and -challenged mice was similar to that of day 32 in the standard protocol. Beyond this time point, AR decreased, approximately reaching control levels on days 39 and 44. Lungs of PBS-PBS and Ova-PBS mice did not show alterations of their responsiveness at any time point tested (Fig. 5A).

Ex vivo AR to intravascular MCh in IPML: correlation to AR in vivo. Data of the time course experiments were pooled, and ex vivo AR values were correlated with P_{enh} data of the respective intact mice. The fold airway resistance values of the IPML ($n = 60$) correlated significantly with P_{enh} values (Fig. 5B). A detailed analysis showed significant correlations for the two early time points after sensitization and challenge (day 31/32 and day 34/35, respectively; Fig. 5, C and D). At later time points, no significant correlations were observed, apparently because of the overall low MCh response, which was indistinguishable among groups (data not given in detail).

Fenoterol diminished AR in IPML. Testing the sensitivity of the AHR in IPML to bronchodilators, lungs of sensitized and challenged mice were perfused with increasing doses of fenoterol (Fig. 6). In the absence of fenoterol, lungs of Ova-Ova mice showed distinctively enhanced AR after intravascular MCh provocation compared with PBS-PBS lungs. Fenoterol at a dose of 0.01 $\mu\text{g}/\text{ml}$ in Ova-Ova lungs reduced AR to levels of fenoterol-free perfused Ova-PBS and PBS-PBS lungs. Increasing the doses of fenoterol further reduced the AR in the isolated lungs in a dose-dependent manner. In lungs of PBS-PBS and Ova-PBS mice, AR was reduced significantly and dose-dependently by fenoterol as well.

DISCUSSION

The present study demonstrates that ex vivo perfused and ventilated lungs of mice sensitized and challenged with allergen display marked AHR, which correlates well with noninvasive measurements in spontaneously breathing mice.

In recent years, mouse models of allergic lung inflammation have offered new investigational opportunities (8). The IPML utilizes the advantages of an intact organ with functional integrity, which is independent from any impact of other organs, allows accurate measurement of lung function parameters, and makes the vascular and alveolar space easily accessible, thereby providing new possibilities to analyze pathogenetic mechanisms.

To study AR to aerosolized MCh in IPML, we used a customized aerosolization system that allowed effective delivery of aqueous solutions into the airways without affecting airway resistance per se. When provoked with aerosolized MCh, lungs of sensitized and challenged mice showed explicitly higher elevations of airway resistance compared with controls. Furthermore, murine in vivo AR, measured by means of WBP 1 day before the IPML procedure, strongly correlated with AR determined in the respective isolated organ. The WBP technique determines the increase of the dimensionless parameter P_{enh} as an index of increased AR (14). Although this parameter does not directly represent changes in airway diameter or resistance and is therefore not a substitute for established parameters of lung mechanics, it reflects mechanical changes of the airways following respiratory distress (28). It therefore seems to be most valuable in models with predominant bronchial hyperresponsiveness due to peribronchial rather than parenchymal inflammation, such as allergen-induced AHR (9–11, 13, 24). To directly compare AR detected in isolated lungs with data from in vivo techniques using intact animals, we chose WBP as the least invasive form of lung function measurements (3). Our findings showed a high degree of correlation between the level of AHR in the two techniques, suggesting that isolation, ventilation, and perfusion of the lungs did not significantly alter ex vivo AHR of mice sensitized and challenged with allergen. In regard to the controversy about the dimensionless parameter P_{enh} (1, 3, 28), we performed another set of experiments sequentially measuring lung function in the same mice first by noninvasive WBP (P_{enh}), then by invasive measurement of airway resistance in intubated and spontaneously breathing mice, and finally by assessing airway resistance ex vivo in IPML. Our results in BALB/c mice showed a significant correlation between MCh-induced increases in P_{enh} values and increases in airway resistance obtained in intubated mice as well as in isolated lungs, pointing toward the validity of the data comparing WBP and IPML.

Alternatively to inhalative MCh provocation, intravenous application has been used in vivo for the assessment of murine AR (19, 27). Whereas intravenous dosage in mice is technically demanding and accompanied by transient increases in blood volume and potentially by severe cardiac side effects (2) in vivo, vascular concentrations of drugs and hemodynamic parameters can be controlled easily in IPML. When perfused with increasing MCh buffer concentrations, lungs of allergen-sensitized and -challenged mice responded dose-dependently and showed enhanced AR compared with control lungs in our model. These results correlated closely with those derived from

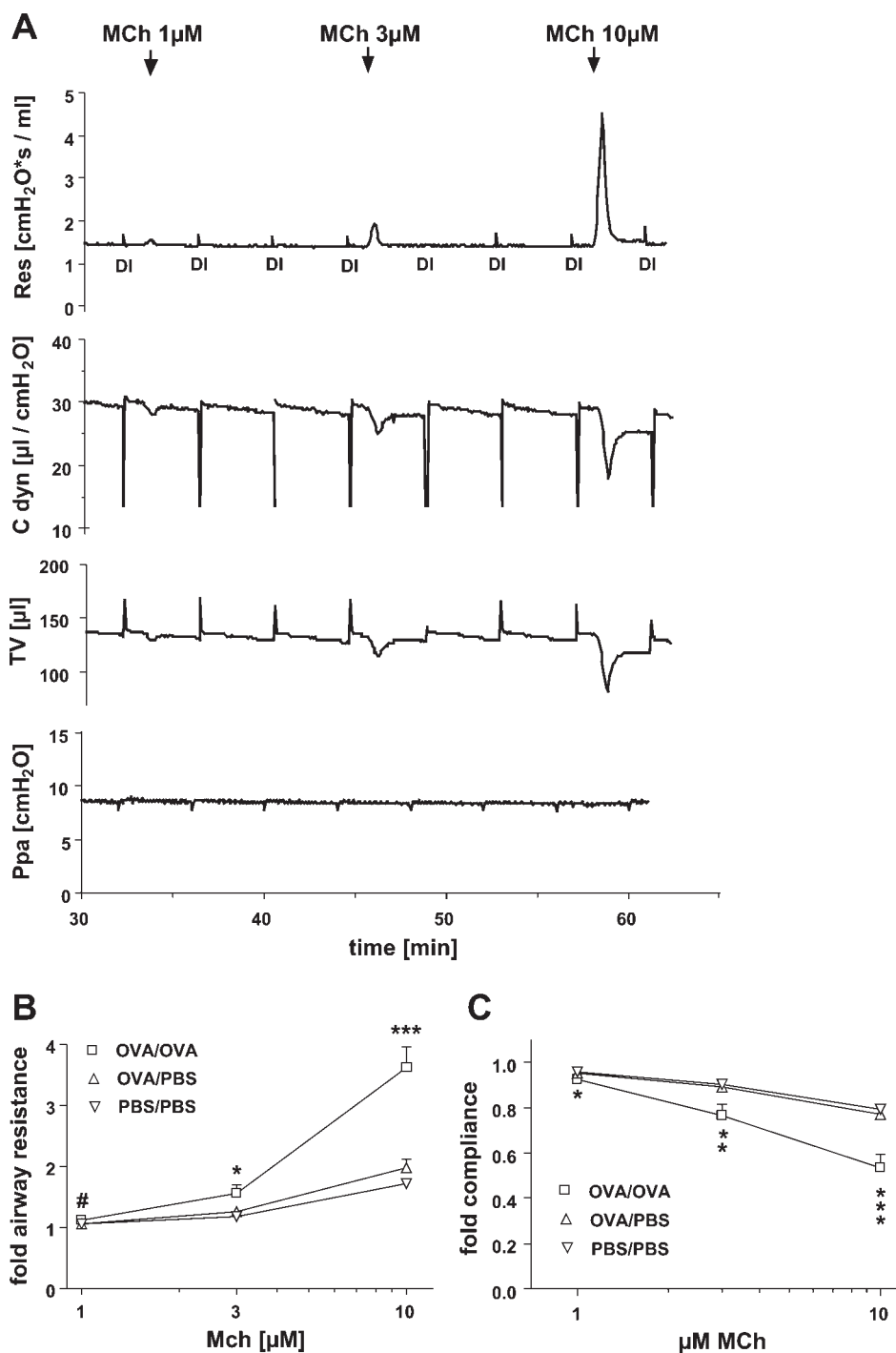


Fig. 4. AR to intravascular MCh provocation in isolated mouse lungs. Mice were allergen sensitized and challenged. Perfusion of the isolated lung was repetitively changed to buffer containing MCh (30 s each dose), and AR was determined. A: original registration of single experiment (Ova-Ova). MCh-induced changes of airway resistance (B) and dynamic compliance (C) were expressed as fold change. Res, airway resistance; C_{dyn}, dynamic compliance; TV, tidal volume; P_{pa}, pulmonary arterial pressure; DI, deep inspiration. Values are means $\pm$ SE ($n = 8$ each). Error bars are not shown when symbols are larger than bars. # $P < 0.05$ vs. PBS-PBS; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. Ova-PBS and PBS-PBS.

WBP and suggest that MCh perfusion is an adequate alternative for detection of mouse lung hyperresponsiveness.

It is known that hyperreactivity following Ova exposure resolves after cessation of allergen challenge (8). Accordingly, ex vivo AHR determined by intravascular MCh persisted for at least 5 days after the last Ova challenge (day 35 of the protocol) and had returned to baseline levels on days 39 and 44. On days 32 and 35, these results showed good correlation with in vivo AR measurements by P_{enh} . A comparable time course of AHR persistence was previously detected by WBP in mice (8).

There are only a few studies addressing allergen-induced airway responses in Ova-exposed intact mice, showing reactions of varying strength (5, 6, 30). These early-phase reactions following specific provocation may result from the release of inflammatory mediators leading to bronchoconstriction as well as airway edema. We demonstrated here in lungs of sensitized and challenged mice that ex vivo aerosolized Ova induced increased early-phase responses, reaching a maximum at 10–30 min after allergen inhalation. Although the rise in airway resistance was moderate under the current experimental conditions, these data showed that

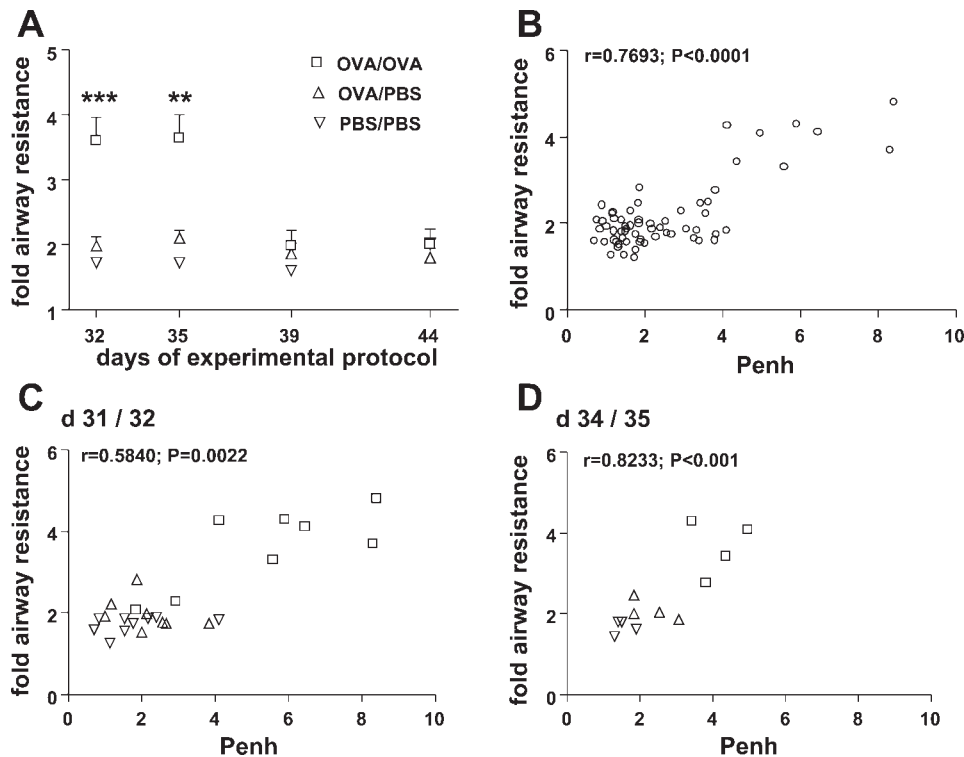


Fig. 5. Temporal kinetics of AR after allergen sensitization and challenge. Mice were allergen sensitized and challenged. One, four, eight, or thirteen days after the last challenge (i.e., day 31, 34, 38, or 43 of protocol), AR was measured in intact mice by determination of P_{enh} ($n = 8$ /group for day 32, $n = 4$ for all other time points and groups; total 60 mice). Correspondingly, AR was measured 24 h later (i.e., 2, 5, 9, or 14 days after the last challenge at day 30) in their perfused lungs. AR was determined considering values 30 s before MCh perfusion and at maximum airway resistance. **A**: temporal kinetics of AR measured in isolated lungs; $n = 8$ /group for day 32, $n = 4$ for all other time points and groups. Values are means $\pm$ SE. Error bars are not shown when symbols are larger than bars. $**P < 0.01$, $***P < 0.001$ vs. Ova-PBS and PBS-PBS. **B-D**: correlation between AR in unrestrained mice after MCh inhalation and AR in their isolated lungs after MCh infusion, analyzed in total (**B**) and for different time points as indicated (**C** and **D**). **d**, Day. Data were analyzed with Spearman's correlation test; P values are 2 tailed.

allergen-induced airway responses are preserved even in isolated lungs.

In the IPML model, airway resistance and dynamic compliance, which are considered the gold standard parameters for AR studies in mice (7), were continuously monitored, as were tidal volume and peak inspiratory and expiratory flow. Baseline values of respiratory parameters of IPML were similar to those reported for anesthetized, mechanically ventilated mice (15). The bronchial and vascular reactivity of naive lungs in response to diverse agonists including MCh has been characterized previously in this model (16, 17, 29). The allergen

sensitization and challenge procedure did not affect baseline airway resistance values of isolated lungs, which is in accordance with in vivo studies (15).

Acute elevation of pulmonary venous pressure has been reported to induce airway wall thickening (4, 32) and may thereby contribute to altered bronchoconstriction and -relaxation (32, 34). However, venous pressure elevation did not cause changes either in baseline resistance or in AR of sensitized or naive lungs in response to intravenous MCh (data not shown in detail). Furthermore, changes in end-expiratory pressure between 2 and 7 cmH₂O during negative-pressure ventilation did not affect AR in our model (data not given in detail). Nevertheless, pulmonary venous pressure and ventilation parameters were kept constant in all experiments.

When lungs were perfused with the β_2 -agonist fenoterol, the MCh response was dose-dependently diminished, indicating that the response to bronchodilators is preserved in the isolated organ. The increase of airway resistance was not completely abolished by fenoterol but consistently decreased to comparable levels in Ova-Ova and control mice.

In conclusion, ex vivo perfused and ventilated lungs from allergen-sensitized and -challenged mice displayed marked and reproducible AHR, which correlated well with alterations of AR measured by two different techniques in vivo. Because the basic features of AHR were preserved in IPML, a more analytical approach can be used to delineate mechanisms of AHR. In addition, this model provides new opportunities to analyze pharmacological interventions at an intact organ level.

GRANTS

This work was supported in part by grants from the Bundesministerium für Bildung und Forschung to S. Rosseau and N. Suttrop (CAPNETZ—competence network community acquired pneumonia), from Charité-Universitäts-

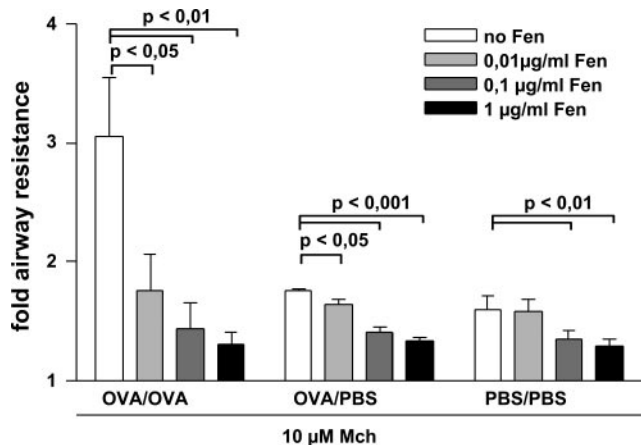


Fig. 6. AR is reduced by fenoterol (Fen) perfusion. Mice were allergen sensitized and challenged. Isolated lungs were perfused and ventilated continuously. Intravascular MCh provocation (10 μ M) was performed at 12-min intervals for 30 s each. Fenoterol was infused with increasing concentrations starting 9 min before each MCh provocation. AR was determined considering values 30 s before MCh perfusion and at maximum airway resistance; $n = 4$ each.

medizin Berlin to M. Witzernath and H. Schütte, and from Deutsche Forschungsgemeinschaft [SFB587 (Z2)] to A. Braun and H. G. Hoymann.

REFERENCES

- Adler A, Cieslewicz G, and Irvin CG. Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *J Appl Physiol* 97: 286–292, 2004.
- Ameredes BT. Cardiac activity during airway resistance alterations with intravenous and inhaled methacholine. *Respir Physiol Neurobiol* 139: 281–292, 2004.
- Bates JH and Irvin CG. Measuring lung function in mice: the phenotyping uncertainty principle. *J Appl Physiol* 94: 1297–1306, 2003.
- Brown RH, Zerhouni EA, and Mitzner W. Visualization of airway obstruction in vivo during pulmonary vascular engorgement and edema. *J Appl Physiol* 78: 1070–1078, 1995.
- Carey MA, Germolec DR, Bradbury JA, Gooch RA, Moorman MP, Flake GP, Langenhach R, and Zeldin DC. Accentuated T helper type 2 airway response after allergen challenge in cyclooxygenase-1^{-/-} but not cyclooxygenase-2^{-/-} mice. *Am J Respir Crit Care Med* 167: 1509–1515, 2003.
- Cieslewicz G, Tomkinson A, Adler A, Duez C, Schwarze J, Takeda K, Larson KA, Lee JJ, Irvin CG, and Gelfand EW. The late, but not early, asthmatic response is dependent on IL-5 and correlates with eosinophil infiltration. *J Clin Invest* 104: 301–308, 1999.
- Drazen JM, Finn PW, and De Sanctis GT. Mouse models of airway responsiveness: physiological basis of observed outcomes and analysis of selected examples using these outcome indicators. *Annu Rev Physiol* 61: 593–625, 1999.
- Epstein MM. Do mouse models of allergic asthma mimic clinical disease? *Int Arch Allergy Immunol* 133: 84–100, 2004.
- Finotto S, De Sanctis GT, Lehr HA, Herz U, Buerke M, Schipp M, Bartsch B, Atreya R, Schmitt E, Galle PR, Renz H, and Neurath MF. Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. *J Exp Med* 193: 1247–1260, 2001.
- Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, Ackerman K, Haley K, Galle PR, Szabo SJ, Drazen JM, De Sanctis GT, and Glimcher LH. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 295: 336–338, 2002.
- Gerhold K, Bluemchen K, Franke A, Stock P, and Hamelmann E. Exposure to endotoxin and allergen in early life and its effect on allergen sensitization in mice. *J Allergy Clin Immunol* 112: 389–396, 2003.
- Glaab T, Mitzner W, Braun A, Ernst H, Korolewicz R, Hohlfeld JM, Krug N, and Hoymann HG. Repetitive measurements of pulmonary mechanics to inhaled cholinergic challenge in spontaneously breathing mice. *J Appl Physiol* 97: 1104–1111, 2004.
- Hamada K, Goldsmith CA, Goldman A, and Kobzik L. Resistance of very young mice to inhaled allergen sensitization is overcome by coexposure to an air-pollutant aerosol. *Am J Respir Crit Care Med* 161: 1285–1293, 2000.
- Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, and Gelfand EW. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 156: 766–775, 1997.
- Hashimoto K, Peebles RS Jr, Sheller JR, Jarzecka K, Furlong J, Mitchell DB, Hartert TV and Graham BS. Suppression of airway hyperresponsiveness induced by ovalbumin sensitization and RSV infection with Y-27632, a Rho kinase inhibitor. *Thorax* 57: 524–527, 2002.
- Held HD, Martin C, and Uhlig S. Characterization of airway and vascular responses in murine lungs. *Br J Pharmacol* 126: 1191–1199, 1999.
- Held HD and Uhlig S. Basal lung mechanics and airway and pulmonary vascular responsiveness in different inbred mouse strains. *J Appl Physiol* 88: 2192–2198, 2000.
- Larsen GL, Renz H, Loader JE, Bradley KL, and Gelfand EW. Airway response to electrical field stimulation in sensitized inbred mice. Passive transfer of increased responsiveness with peribronchial lymph nodes. *J Clin Invest* 89: 747–752, 1992.
- Leigh R, Southam DS, Ellis R, Wattie JN, Sehmi R, Wan Y, and Inman MD. T-cell-mediated inflammation does not contribute to the maintenance of airway dysfunction in mice. *J Appl Physiol* 97: 2258–2265, 2004.
- Martin TR, Gerard NP, Galli SJ, and Drazen JM. Pulmonary responses to bronchoconstrictor agonists in the mouse. *J Appl Physiol* 64: 2318–2323, 1988.
- National Heart, Lung, and Blood Institute and GINA Executive Committee. *Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention*. NIH Publication No. 02-3659, 2004.
- Neuhaus-Steinmetz U, Glaab T, Daser A, Braun A, Lommatzsch M, Herz U, Kips J, Alarie Y, and Renz H. Sequential development of airway hyperresponsiveness and acute airway obstruction in a mouse model of allergic inflammation. *Int Arch Allergy Immunol* 121: 57–67, 2000.
- O'Byrne PM and Inman MD. Airway hyperresponsiveness. *Chest* 123: 411S–416S, 2003.
- Park JK, Kim YK, Lee SR, Cho SH, Min KU, and Kim YY. Repeated exposure to low levels of sulfur dioxide (SO₂) enhances the development of ovalbumin-induced asthmatic reactions in guinea pigs. *Ann Allergy Asthma Immunol* 86: 62–67, 2001.
- Postma DS and Kerstjens HA. Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 158: S187–S192, 1998.
- Renz H, Smith HR, Henson JE, Ray BS, Irvin CG, and Gelfand EW. Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. *J Allergy Clin Immunol* 89: 1127–1138, 1992.
- Schuh JM, Power CA, Proudfoot AE, Kunkel SL, Lukacs NW, and Hogaboam CM. Airway hyperresponsiveness, but not airway remodeling, is attenuated during chronic pulmonary allergic responses to *Aspergillus* in CCR4^{-/-} mice. *FASEB J* 16: 1313–1315, 2002.
- Schwarze J, Hamelmann E, Gelfand EW, Adler A, Cieslewicz G, and Irvin CG. Barometric whole body plethysmography in mice. *J Appl Physiol* 98: 1955–1957, 2005.
- Seybold J, Thomas D, Witzernath M, Boral S, Hocke AC, Bürger A, Hatzelmann A, Tenor H, Schudt C, Krüll M, Schütte H, Hippenstiel S, and Suttrop N. Tumor necrosis factor- α -dependent expression of phosphodiesterase 2: role in endothelial hyperpermeability. *Blood* 105: 3569–3576, 2005.
- Trifilieff A, El Hasim A, Corteling R, and Owen CE. Abrogation of lung inflammation in sensitized Stat6-deficient mice is dependent on the allergen inhalation procedure. *Br J Pharmacol* 130: 1581–1588, 2000.
- von Bethmann AN, Brasch F, Nusing R, Vogt K, Volk HD, Müller KM, Wendel A, and Uhlig S. Hyperventilation induces release of cytokines from perfused mouse lung. *Am J Respir Crit Care Med* 157: 263–272, 1998.
- Wagner EM. Effects of edema on small airway narrowing. *J Appl Physiol* 83: 784–791, 1997.
- Wanner A, Abraham WM, Douglas JS, Drazen JM, Richerson HB, and Ram JS. NHLBI Workshop Summary. Models of airway hyperresponsiveness. *Am Rev Respir Dis* 141: 253–257, 1990.
- Yager D, Kamm RD, and Drazen JM. Airway wall liquid. Sources and role as an amplifier of bronchoconstriction. *Chest* 107: 105S–110S, 1995.