Detection of allergen-induced airway hyperresponsiveness in isolated mouse lungs

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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; BvVG, Berlin, Germany) were maintained on an ovalbumin (Ova)-free diet. Systemic sensitization with Ova (20 μg/injection; Sigma, Deisenhofen, Germany) adsorbed to 2 mg of Al(OH)3 (Pierce, Rockford, IL) by intraperitoneal injections on days 28, 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).

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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; Fa¨hrhofer ITEM; licensed by Buxco, Troy, NY). Aerosol concentrations were 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) as described previously (14). Briefly, aerosol was continuously added to the nebulizer’s output. Inhalative MCh concentration 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-hydroxyethylamylopectin 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 β2-agonist fenoterol were continuously added to the buffer fluid. AR was determined as described above.

Data analysis. Data are expressed as means ± 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·s·ml−1; compliance: Ova-Ova 37.10 ± 2.05, Ova-PBS 39.74 ± 3.83, PBS-PBS 40.19 ± 2.76 μl/cmH2O), 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).
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-PBS.
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 μg/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.
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 \( P_{aw} \). 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
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 β2-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.

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