Basal lung mechanics and airway and pulmonary vascular responsiveness in different inbred mouse strains

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Held, Heinz-Dieter, and Stefan Uhlig. Basal lung mechanics and airway and pulmonary vascular responsiveness in different inbred mouse strains. J. Appl. Physiol. 88: 2192–2198, 2000.—Little is known about interstrain variations in baseline lung functions or smooth muscle contractility in murine lungs. We therefore examined basal lung mechanics and airway, as well as vascular reactivity to methacholine, thromboxane (using U-46619, and endothelin-1 (ET-1), A/J, AKR, BALB/c, C3H/HeN, C57BL/6, and SCID mice. All experiments were performed with isolated perfused mouse lungs. Except AKR mice (which were excluded from further analysis), all other strains showed stable pulmonary compliance, pulmonary resistance, and pulmonary arterial pressure within a control period of 45 min. Among these strains, C3H/HeN mice exhibited higher dynamic pulmonary compliance and lower pulmonary resistance, whereas SCID mice had higher baseline pulmonary resistance than the other strains. Concentration-response experiments with methacholine showed a lower airway reactivity for C57BL/6 mice compared with the other strains. Perfusion with 1 μM U-46619 or 100 nM ET-1 revealed a similar pattern: the agonist-inducible broncho- and vasoconstriction was lower in C57BL/6 mice than in all other strains, whereas it tended to be higher in SCID mice. The present study demonstrates a correlation between airway and vascular responsiveness in all tested strains. SCID mice are hyperreactive, whereas C57BL/6 mice are hyporeactive, to smooth muscle constrictors. Lung mechanics, as well as airway and vascular responsiveness, appear to be genetically controlled.

perfusion mouse lung; hyperresponsiveness; genetic control; endothelin; thromboxane

WITH THE GROWING AWARENESS of the fact that chronic or acute inflammation is pivotal to the understanding of many lung diseases, the interest in interactions between the immune system and lung functions continually rises. Because the immune system of the mouse is well defined, many new mouse-based models of pulmonary diseases are currently being developed. To be able to analyze lung functions in mice, we have developed the model of the isolated perfused and ventilated mouse lung, which enables the simultaneous assessment of airway and pulmonary vascular mechanics in this species (24). Recently, using BALB/c mice, we characterized the effects of various endogenous pressor agents in this model (9).

Many different mouse strains are available, and knowledge of interstrain variations is important both from a genetic point of view as well as from a more practical one when setting up a new model. This prompted us to investigate basal lung mechanics and the reactivity toward various broncho- and vasoconstrictors in six inbred mouse strains, all of which have been used in the investigation of inflammatory lung diseases.

MATERIALS AND METHODS

Animals

Male A/J, AKR, BALB/c, C3H/HeN, C57BL/6, and SCID mice were obtained from Charles River (Sulzfeld, Germany). All animals were used at a weight of 22–27 g, except C57BL/6 mice, which weighed 22–35 g. The baseline values for pulmonary compliance and pulmonary resistance or the reactivity toward methacholine, U-46619, or endothelin-1 (ET-1) did not depend on the animal weight.

Materials

Pentobarbital sodium (Nembutal) was purchased from the Wirtschaftsgenossenschaft Deutscher Tierärzte (Hannover, Germany), bovine albumin low endotoxin grade (lot 08152) from Serva (Heidelberg, Germany), ET-1 from Boehringer Mannheim (Mannheim, Germany), U-46619 from Cayman (Ann Arbor, MI), ET-1 from Boehringer Mannheim (Mannheim, Germany), and RPMI 1640 from Bio Whittaker (Verviers, Belgium).

Isolated Perfused Mouse Lung Preparation

The mouse lungs were prepared and perfused essentially as described recently (24). Briefly, lungs were perfused in a nonrecirculating fashion through the pulmonary artery at a constant flow of 1 ml/min, resulting in a pulmonary arterial pressure (Ppa) of −0.5 to −5 cmH2O. For technical reasons (to minimize edema formation and reduce costs of perfusate), the chosen perfusate flow rate was only −1/10 the physiological flow rate (8). However, relative comparisons between different mouse strains are still feasible under these conditions. As a perfusion medium, we used RPMI medium lacking phenol red (37°C) that contained 4% low endotoxin grade albumin. The lungs were ventilated by negative pressure (−3 to −9 cmH2O) with 90 breaths/min, resulting in a tidal volume of ~200 µl. A hyperinflation (−20 to −25 cmH2O) was performed every 5 min. As previously shown at the light-microscopical level, this setup does not cause pulmonary/alveolar edema or other structural changes to the lungs within the time studied here (24).

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Artificial thorax chamber pressure was measured with a differential pressure transducer (Validyne DP 45–24, Northridge, CA), and air flow velocity was measured with a pneumotachograph tube connected to a differential pressure transducer (Validyne DP 45–15). The arterial pressure was continuously monitored by means of a pressure transducer (Isotec Healthdyne, Irvine, CA) that was connected with the cannula ending in the pulmonary artery. All data were transmitted to a computer and analyzed by the Pulmodyn software (Hugo Sachs Elektronik, March Hugstetten, Germany). For lung mechanics, the data were analyzed by applying the following formula

$$P = \frac{1}{C} V + R_L \frac{dV}{dt}$$

where $P$ is chamber pressure, $C$ is pulmonary compliance, $V$ is tidal volume, and $R_L$ is pulmonary resistance. The measured pulmonary resistance was corrected for the resistance of the pneumotachometer and the tracheal cannula of 0.6 cmH$_2$O·s·ml$^{-1}$.

**Experimental Design**

After preparation, the lungs from 9 to 10 animals of each strain were perfused and ventilated for 45 min without any treatment to obtain a baseline. Subsequently, three to four lungs each were used to study separately the responsiveness to methacholine, U-46619, or ET-1. When repetitive concentrations of methacholine (10 nM–100 µM) were administered (3–4 animals for each strain), each concentration was given for 20 min, followed by intervals of 15 min in which the lungs were perfused with buffer only (total experiment duration 240 min). U-46619 and ET-1 do not give reproducible results when given more than once to the same lung (9). Therefore, both U-46619 and ET-1 were perfused in separate experiments (3–4 animals for each strain) for 30 min with concentrations of 1 µM and 100 nM, respectively (total experiment duration of 90 min).

**Statistics**

The baseline data for pulmonary resistance (log-transformed because of heteroscedasticity) and compliance were analyzed by ANOVA followed by Tukey's post test. The concentration-response curves for methacholine-induced bronchoconstriction were calculated and compared with Allfit (2) on the basis of the mean increase in pulmonary resistance of each concentration within the perfusion period of 20 min. The statistical groupings for all other parameters were based on the area under the curve (AUC) of a 30-min time interval and were analyzed by ANOVA followed by the Tukey-Kramer test (Graphpad Prism 2.01, Graphpad Software, San Diego, CA). To further analyze the pulmonary smooth muscle responsiveness, a three-way ANOVA (JMP 3.2.2, SAS Institute, Cary, NC) was used to compare all strains simultaneously and to separate the strain effects from those caused by the different treatments (methacholine, U-46619, ET-1) and the related parameters (pulmonary resistance, Ppa). In this analysis, we were only interested in the pressor responses to identify hypo- or hyperreactive strains; therefore, the effect of methacholine on Ppa (which is relaxant) was not included in the analysis. Subsequent to the three-way ANOVA, each mouse strain was tested against all other strains, and the alpha level of these multiple comparisons was adjusted according to the Hommel procedure (see Ref. 27).

**RESULTS**

**Basal Lung Functions**

The baseline values for pulmonary compliance and pulmonary resistance after perfusion and ventilation under control conditions for 45 min are shown in Fig. 1. C3H/HeN mice showed higher compliance than all other strains and had lower pulmonary resistance. SCID mice exhibited significantly higher pulmonary resistance than all other strains but comparable lung compliance. AKR mice showed continuously decreasing compliance and increasing pulmonary resistance during the control period. Therefore, this strain was excluded from further experiments. Baseline Ppa did not vary between the strains (data not shown).

**Reactivity to Broncho- and Vasocostricators**

Methacholine. The concentration-dependent increase in pulmonary resistance after challenge with the stable acetylcholine derivate methacholine is shown in Fig. 2. C57BL/6 mice showed significantly less maximal bron-
choconstriction than all other strains, whereas the values for concentration at which half-maximal bronchoconstriction was achieved (EC50) did not vary among the different strains (logEC50 ± SE: 2.50 ± 0.68 for the pooled data). It should be noted, however, that for two strains (BALB/c and C3H/HeN) a plateau might not have been reached at 10−4 M methacholine. Therefore, we cannot exclude the possibility that the EC50 values for these two strains are higher than the value given above.

U-46619. Figure 3 shows the time course and statistical grouping of broncho- and vasoconstriction elicited by the stable thromboxane-receptor agonist U-46619. Whereas SCID mice exhibited a marked broncho- and vasoconstrictory response to U-46619 (1 µM), C57BL/6 mice were less reactive, although the difference did not reach statistical significance for the airways. Statistical analysis of U-46619-elicited vasoconstriction resulted in overlapping groups, i.e., SCID mice were significantly different from C3H/HeN and C57BL/6 mice, whereas BALB/c and A/J mice were only different from C57BL/6 mice.

ET-1. Challenging murine lungs with ET-1 (10 nM) resulted in a pattern of airway and vascular reactivity (Fig. 4) similar to that observed after perfusion with U-46619. Again, C57BL/6 mice showed a weaker bronchoconstriction than all other strains. Differences in ET-1-elicited vasoconstriction were less pronounced, but again BALB/c, SCID, and A/J mice reacted stronger than C57BL/6 mice.

Fig. 2. Methacholine-induced bronchoconstriction in different mouse strains. The different concentrations of methacholine (cMCh) were perfused for 20 min, with intervals of 15 min in which lungs were perfused with buffer only. Capital letters indicate the statistical groupings, i.e., different capital letters indicate groups that were significantly different from all other groups tested at P < 0.05. Data are means ± SE of 3 mice.

Fig. 3. Time course (A and B) and statistical grouping (C and D) of broncho- (A and C) and vasoconstriction (B and D) elicited by 1 µM U-46619 in different mouse strains. Data were analyzed by Tukey-Kramer test for multiple comparisons, based on the area under the curve (AUC) of each treatment. PAP, pulmonary arterial pressure. Capital letters indicate statistical groupings, i.e., different capital letters indicate groups that were significantly different from all other groups tested at P < 0.05. Data are given as means ± SE; n = 3–4.
Statistical analysis. A three-way ANOVA on all responsiveness data was performed to separate the strain effects from those caused by the treatments (methacholine, U-46619, ET-1) and the related parameters (Ppa, pulmonary resistance). There was a significant effect of the strains ($P < 0.0001$), which was further analyzed by contrasting each strain against all others. Two strains were significantly different from all others: C57BL/6 mice were hyporeactive ($P = 0.0002$ vs. all others), whereas SCID mice were hyperreactive ($P = 0.0016$ vs. all others). BALB/c ($P = 0.0846$), C3H/HeN ($P = 0.2848$), and A/J mice ($P = 0.4548$) were not different from the other strains. In addition, when using the mean AUCs calculated for U-46619 and ET-1 treatment, there was a good correlation between airway and vascular reactivity among all strains (Fig. 5).

DISCUSSION

This study provides information about baseline lung functions, as well as airway and pulmonary vascular responsiveness, of BALB/c, C3H/HeN, C57BL/6, A/J, and SCID mice. The model of the isolated, perfused, and ventilated mouse lung that was used here is, at present, the only model that allows simultaneous assessment of lung mechanics and Ppa in mice. Therefore, this study differs from previous studies on mouse strains not only in that mediators other than serotonin or methacholine were studied but more importantly also in the fact that for the first time the responsiveness of the pulmonary artery was compared with that of the pulmonary vein.

Fig. 4. Time course (A and B) and statistical grouping (C and D) of broncho- (A and C) and vasoconstriction (B and D) elicited by 100 nM endothelin-1 in different mouse strains. Data were analyzed by Tukey-Kramer test for multiple comparisons, based on the AUC of each treatment. Capital letters indicate statistical groupings, i.e., different capital letters indicate groups that were significantly different from all other groups tested at $P < 0.05$. Data are given as means ± SE; $n = 3–4$.

Fig. 5. Correlation between airway and vascular reactivity. Plotted are mean vascular responses vs. mean airway responses of lungs from different mouse strains exposed to either U-46619 (closed symbols) or endothelin-1 (open symbols). $\triangle$ and $\circ$, BALB/c; $\star$ and $\diamond$, C57BL/6; $\mathbf{\Delta}$ and $\Delta$, A/J; $\mathbf{\bullet}$ and $\circ$, C3H/HeN; $\mathbf{\nabla}$ and $\nabla$, SCID; $n = 3$. Pearson’s correlation coefficient calculated between airway and vascular responses was 0.74 ($P = 0.015$).
airways. The good correlation between vascular and airway responsiveness (Fig. 5) suggests that both types of smooth muscle are controlled by similar mechanisms.

The strains were selected according to their usage in lung physiological and immunologic investigations: BALB/c and C3H/HeN mice are strains frequently used in laboratory animal research, and many immunologic examinations have utilized these strains. C57BL/6 mice were included because this strain serves as the background strain of many genetically engineered mice. A/J and AKR mice were selected because previous studies reported them to be more sensitive to mediators such as acetylcholine or platelet activating factor (14, 15) than other strains. Finally, the SCID mouse was tested because its lack of lymphocytes enables investigation of the role of the adaptive immune response in various inflammatory or infectious lung disorders.

Previous investigations on the breathing frequency, tidal volume, and static lung compliance of C3H/HeJ mice and C57BL/6J mice already suggested that differences in lung functions between different mouse strains exist and that they are subject to genetic control (20–22). Another series of studies was initiated by the observation that, among a number of inbred mouse strains investigated in vivo, the bronchopulmonary response to acetylcholine was considerably greater in A/J mice compared with C3H mice (5–7, 13). However, these studies did not address pulmonary resistance (except Ref. 5), dynamic pulmonary compliance (except Ref. 5), or the pulmonary vasculature. Our data show that, among the most commonly used mouse strains, differences exist with respect to their basal physiological lung properties, as well as their responsiveness to methacholine, thromboxane, or endothelin.

Basal Lung Physiology

In the perfused mouse lung, only the AKR mouse failed to become stable with respect to baseline lung mechanics (pulmonary resistance and dynamic compliance) within the control period. All other strains could be examined in the perfused mouse lung model for at least 240 min. Of these, C3H/HeN mice showed significantly higher dynamic compliance compared with all other strains. This is in line with a slow, deep-breathing phenotype in the C3H/HeJ strain, compared with a rapid, shallow-breathing pattern in C57BL/6 mice (21). In addition, compared with C57BL/6 mice, C3H/HeJ mice are known to have a higher static compliance (22). Our data further show that the increased compliance in C3H mice is accompanied by a lower baseline pulmonary resistance than that of all other strains tested here. This is in line with a previous study that showed a lower pulmonary resistance in C3H mice compared with A/J mice in vivo (5).

SCID mice, on the other hand, exhibited higher pulmonary resistance than the other strains tested (except AKR). Interestingly, the dynamic pulmonary compliance of these mice did not differ from, e.g., BALB/c mice, suggesting either that lung compliance and pulmonary resistance are under the control of different genetic factors or, alternatively, that lymphocytes control baseline pulmonary resistance by an unknown mechanism. Evidence for the latter hypothesis is provided by the fact that T lymphocytes have been shown to modulate airway responsiveness (3).

The absolute values of pulmonary resistance in the present study are at the lower range of previously reported data from in vivo studies. Because most available literature data are from C57BL/6 mice, we will focus on this strain. The baseline value for pulmonary resistance of C57BL/6 mice in this study was \(0.30 \pm 0.07\) cmH\(_2\)O·s·ml\(^{-1}\). Previously reported baseline data (in cmH\(_2\)O·s·ml\(^{-1}\)) for this strain were 0.31 (23), 0.34 (11), 0.43 (1), 0.43 (4), 0.63 (16), 0.77 (10), and 0.83 (26). The reasons for these fluctuations in baseline airway resistance among different studies are not known but may be related to the technical difficulties of measuring airflow velocity in these animals. The absolute values of dynamic pulmonary compliance are in the range of previously reported data, e.g., again in C57BL/6 mice, 0.021 ± 0.004 ml/cmH\(_2\)O in the present study compared with 0.022 ± 0.001 ml/cmH\(_2\)O reported by Martin et al. (16). It should be noted that the chest wall lung compliance in mice is much greater than the pulmonary compliance (22), so that the compliances measured in vivo and in excised lungs are very similar.

Airway and Vascular Responsiveness

Airway and vascular responsiveness was assessed with three agents, i.e., methacholine, the thromboxane-receptor agonist U-46619, and ET-1. Because repetitive application in the same lung is problematic with either U-46619 or ET-1 (9), in the present study, we investigated only a single concentration per lung of the two latter agents. The concentrations chosen were close to the EC\(_{50}\) value determined in BALB/c mice (9), enabling us to detect both hyper- and hyporesponsiveness. With respect to airway and vascular responsiveness, A/J, BALB/c, and C3H/HeN mice behaved similarly. C57BL/6 mice appeared hyporesponsive, whereas SCID mice were hyperresponsive. Our data are supported by previous findings demonstrating a lower airway responsiveness in C57BL/6 mice compared with A/J mice when challenged with acetylcholine, serotonin, or methacholine (12, 14).

Levitt and Mitzner (14) previously showed that A/J mice are much more reactive to acetylcholine compared with BALB/c or C3H/HeJ mice in vivo, a finding that was not made in the present study. The most obvious explanation for these divergent results is differences related to neural or humoral factors that are present in vivo but not in a perfused lung. This conclusion is corroborated by the findings that, in tracheal preparations, no difference in reactivity to carbachol or KCl between A/J mice and C3H mice occurred (25). Of note, the fact that the higher pulmonary resistance at baseline of A/J mice compared with C3H mice occurs both in vivo and in perfused lungs confirms the previous conclusion that the mechanism behind this higher baseline pulmonary resistance in A/J mice is independent of the
mechanism that is responsible for hyperresponsiveness of A/J mice in vivo (25). Properties of the lung tissue that would be maintained in perfused lungs, such as the distribution of the different acetylcholine receptors or of G proteins (7), appear unlikely to directly account for the differences between in vivo and in vitro. The humoral or neural mechanisms involved in the pulmonary hyperresponsiveness of A/J mice to acetylcholine are unknown but have been related to chromosome 6 (6). Recent evidence suggests that interleukin (IL)-9 may be just such a humoral factor that controls airway reactivity, although the gene for IL-9 is located on mouse chromosome 13 (18). Evidence for the T cell cytokine IL-9 includes 1) that T lymphocytes regulate the baseline airway responsiveness to methacholine in C57BL/6 mice, even in the absence of inflammation (3), 2) that the expression of the TH-2 cytokine IL-9 was markedly reduced in hyperreactive C57BL/6 mice (19), and 3) that overexpression of IL-9 caused airway hyperreactivity to methacholine whereas baseline resistance was normal (23). However, because naive IL-9 transgenic mice do not display baseline airway hyperreactivity (17), the precise role of this cytokine in the control of smooth muscle functioning has still to be clarified. Interestingly, in our model, T cell-deficient SCID mice appeared hyperreactive compared with the closely related BALB/c strain, which raises the possibility that T cells, directly or indirectly, might not only positively but also negatively regulate airway responsiveness.

Our data showed a good correlation between airway and vascular responsiveness in all strains; thus, in C57BL/6 mice, both airways and vessels were hyperreactive, and in SCID mice both airways and vessels were hyperresponsive. The observation that the ranking of the airway and vascular responsiveness among the different mouse strains was similar, regardless of the nature of the provoking agent, suggests that genetic differences between the strains do pertain to the contractile apparatus of the smooth musculature rather than to the expression of specific receptors. This does, of course, not exclude the possibility that the difference in the contractile apparatus is regulated by mediators such as cytokines. In addition, however, to the inherent differences in pulmonary responsiveness shown in the present study, additional factors appear to control airway responsiveness in vivo, as suggested by the hyperreactivity of A/J mice that is observed in vivo (13, 14) but not in vitro (this study and Ref. 25).

In summary, C3H/HeN mice exhibited a higher pulmonary compliance and lower pulmonary resistance, whereas SCID mice showed a higher pulmonary resistance. The comparatively similar responsiveness to methacholine, U-46619, and ET-1 suggests that the contractile apparatus of both airway and vascular smooth muscle of C57BL/6 mice is hypersensitive, in contrast to SCID mice, which appear hyperresponsive. This study suggests that genetically determined differences exist in basal lung functions as well as airway and pulmonary vascular responsiveness between various mouse strains.

We thank Doerte Karp for excellent technical assistance. The present study was supported by the Deutsche Forschungsgemeinschaft Grant DFG U 6892-2.

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Received 1 March 1999; accepted in final form 28 January 2000.

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