DOI: 10.1111/all.14681

Use of spirometry-like measurements to monitor house dust mite-induced experimental asthma in mice

To the Editor,

Spirometry is used to diagnose and monitor asthma, a chronic lung disease characterized by respiratory symptoms, airflow limitation and accumulation of immune system cells, including mast cells in the airways. Airway hyperresponsiveness (AHR) measures an increased airway sensitivity and is related to a decline in lung function assessed by spirometry in population-based studies. Spirometry determines the volume and speed of air that a patient can inhale and exhale, which depends on the size of the lung and is modified by lung diseases. Approaches to acquire accurate measurements of spirometry-like parameters in mice utilize negative pressure-driven forced expiration techniques.[1,2] These studies successfully recorded changed lung parameters in chronic obstructive lung disease models, but failed to detect basal changes in spirometry-like parameters for house dust mite (HDM)- or ovalbumin-induced allergic airway inflammation models.

In our study, we investigated the effect of body weight and age on lung function in naïve BALB/c mice using an automated system to compute spirometry-like parameters (Buxco Pulmonary Function Test (PFT), DSI). In this system, the mouse is anesthetized, intubated and connected to a ventilator (methods are provided in Appendix S1). The PFT system uses vacuum to exhaust air (negative pressure) as a surrogate for exhaling and measures the airflow and pressure during a succession of forced pulmonary tests to generate spirometry-like parameters. Weight and age correlated positively with forced expiratory flow in 0.1 seconds (FEV 0.1) (Figure 1A, B). Moreover, peak of expiratory flow (PEF), forced vital capacity (FVC), inspiratory capacity (IC), tidal volume (TV), compliance (Cdyn), vital capacity (VC) and total lung capacity, but not resistance or functional residual capacity correlated positively with weight (Figure S1). When weight-matched female and male mice were analysed, the females were approximately 20 weeks older than the males and showed higher values for most spirometry-like parameters (Figure S2). Most spirometry-like parameters also increased with age in males (Figure S3). Results showing that lung function relates to differences in mouse strain, sex and growth were earlier found using whole-body plethysmography.[3] We conclude that spirometry-like lung function parameters are highly dependent on age and weight, and that great care needs to be taken when matching groups of mice of the same gender for generating this type of data.

To test whether spirometry-like measurements could distinguish mice with HDM-induced lung inflammation from sex-, weight- and age-matched phosphate-buffered saline (PBS) control mice, an established model was used.[4] BALB/c mice received seven intranasal administrations of HDM or PBS on days 0, 3, 6, 9, 12, 15 and 18, and lung function was determined on day 19 (Figure 1C). The mice showed reduced FEV 0.1 and PEF after HDM sensitization (Figure 1E, F). Reductions in FVC, IC, Cdyn and TV were also found in HDM-sensitized mice compared with PBS controls, but no significant reduction in the other parameters was observed (Figure S4). Devos et al recorded spirometry-like parameters in an HDM-model using both forced expiration and forced oscillation technique (FOT) and did not detect basal changes in spirometry-like measurements.[1] However, they used comparably lower HDM doses over a shorter period of time (13 days) and we speculate that this could be why no basal changes were observed. In our study, HDM induced smooth muscle proliferation, and the number of α-actin$^+$ lung cells correlated negatively with FEV 0.1 (Figure S5).

When HDM-sensitized mice were treated with vehicle or dexamethasone before the last four HDM administrations, dexamethasone reduced the number of bronchoalveolar lavage (BAL) eosinophils to a minimum (Figure 1D, Figure S6A), as expected.[5] Dexamethasone improved FEV 0.1, PEF and FVC, and showed a trend ($p = .06$) to improve IC compared with vehicle-treated mice with HDM-induced allergic airway inflammation (Figure 1E-F, Figure S4). Given the tight relationship between poor lung function and AHR, this finding also supports studies demonstrating that dexamethasone prevents AHR in mouse models of allergic inflammation, for example.[5]

Our major interest in mast cells and their role in asthma triggered us to test whether dexamethasone affected mast cells in the lung. Three lung mast cell populations, which differ in integrin β7 expression, exist in HDM-sensitized mice. The mast cell progenitor (MCp) population was the most expanded, followed by the induced mast cells with intermediate integrin β7 expression, which are absent in PBS control mice, and the mature mast cells (Figure 2A). As the anti-FcεRI antibody MAR-1 was shown to cross-react with FcγRI and FcγRIV,[6] the mast cell populations were also quantified using an anti-IgE antibody (Table S1). However, both strategies resulted in a similar quantification (Figure S6D, E).

Dexamethasone reduced the total number of lung mast cells, the MCp and mature mast cell populations, and showed a tendency to decrease the induced mast cells ($p = .07$) (Figure 2A, B). Indeed, asthma patients on inhaled corticosteroids have lower mast cell numbers within the lung epithelium and smooth muscle than those treated with β2-agonist alone.[7] Moreover, glucocorticoids increase the apoptosis of skin mast cells in vivo, likely due to an inhibitory effect on stem cell factor production from fibroblast.[8] Thus, we speculate that the reduction in lung mast cells by dexamethasone is likely due to a combination of reduced influx of MCp and increased apoptosis of lung mast cells. Importantly, not only the mast cell burden, but also mast cell activation was suppressed by dexamethasone. HDM-sensitized mice had twofold increased levels of mouse mast cell protease-1 (mMCP-1) in the BAL fluid, but dexamethasone treatment normalized the levels (Figure 2D). HDM sensitization also induced an increase in FcεRI expression in the lung mast cell populations, which was decreased in mice treated with dexamethasone (Figure 2C). In line with our data, dexamethasone was shown to reduce mast cell expression of FcεRI in vitro.[9] Hence, we speculate that the suppressed mMCP-1 levels in dexamethasone-treated HDM-sensitized mice could be explained by reduced lung mast cell numbers and their abolished activation due to decreased FcεRI.

We previously demonstrated a correlation between a high frequency of human blood MCp and reduced FEV1 and PEF (% of predicted) in patients with allergic asthma.[10] Therefore, we investigated whether a similar association existed in the mouse model of HDM-induced allergic airway inflammation. A high frequency of lung MCp correlated with reduced FEV 0.1 or PEF (Figure 2E, F), and similar relationships were found between BAL eosinophils and FEV 0.1 or PEF (Figure S6B, C). Altogether, our data suggest that a high number of lung MCp predicts reduced lung function.

To summarize, our study provides insight into the relationship between mouse weight/age and spirometry-like pulmonary function parameters in naïve mice, and demonstrates that such parameters can be reduced by allergic airway inflammation, and improved by dexamethasone. However, other allergic airway inflammation models need to be validated to ensure that the spirometry-like signs of reduced lung function are reproduced. To this end, spirometry-like measurements in mice are valid as a pre-clinical tool when investigating new drug targets or disease mechanisms in asthma.

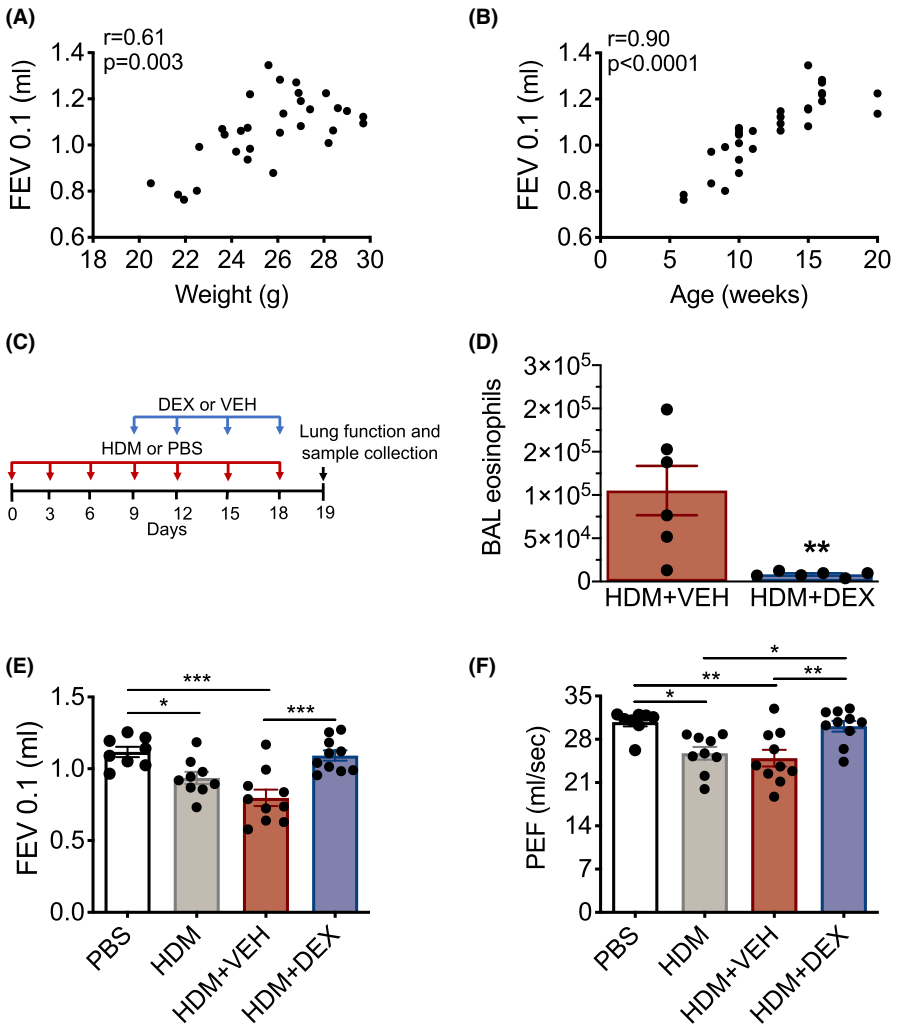

FIGURE 1 Spirometry-like parameters are related to mouse weight, reduced by HDM-induced allergic inflammation, and improved by dexamethasone. (A, B) FEV 0.1 was assessed in naïve mice of different weights (A) and age (B). (C-F) Mice were sensitized with HDM or given PBS as controls. HDM mice received dexamethasone (DEX, 3 mg/kg) or vehicle (VEH, 0.9% NaCl). (D) Eosinophils were quantified in BAL. (E) FEV 0.1 and (F) PEF are shown. Spearman correlation from seven experiments in (A-B). Mean ± SEM from two (D) or three (E-F) independent experiments. Statistical analyses were performed by an unpaired Student's *t* test (D) or one-way ANOVA with post hoc Turkey's test (E-F)

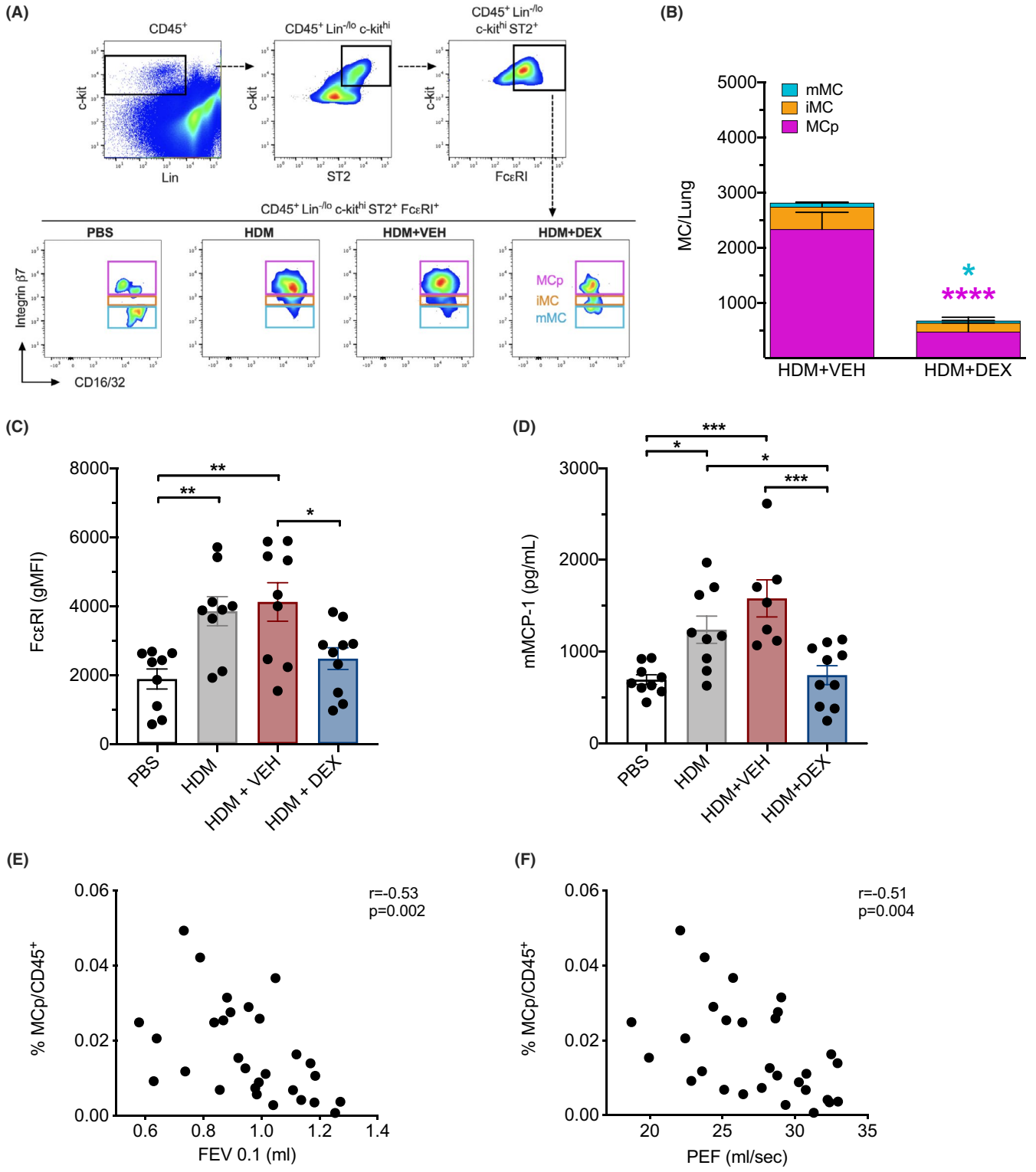

FIGURE 2 Dexamethasone reduces lung mast cell populations, their expression of FcεRI and release of mMCP-1. (A-D) Mice were given PBS or sensitized with HDM alone, or treated with vehicle (HDM + VEH), or dexamethasone (HDM + DEX). (A-C) Mature mast cells (mMC), HDM-induced mast cells (iMC) and MCp were quantified by flow cytometry. (C) Geometric mean fluorescence intensity (gMFI) for FcεRI in lung mast cells. (D) BAL levels of mMCP-1. (E, F) The frequency of lung MCp in treated and untreated HDM mice correlates with FEV 0.1 (E) and PEF (F) using Spearman correlation. (A-F) Data from three independent experiments were pooled. (B-D) The mean ± SEM is shown, and statistical significance was tested by an unpaired Student's *t* test (B) and one-way ANOVA with Turkey's post hoc test (C, D)

ACKNOWLEDGEMENTS

Flow cytometry was performed on equipment provided by the BioVis Facility at the Science for Life Laboratory, Uppsala, Sweden.

KEYWORDS

asthma, house dust mite, lung function, mast cells, mouse model

FUNDING INFORMATION

Hjärt-Lungfonden, Grant/Award Number: 20170479; Knut och Alice Wallenbergs Stiftelse, Grant/Award Number: 2017.0022; Konsul ThC Bergh's Foundation; Ellen, Walter and Lennart Hesselman Foundation for Scientific Research; Consejo Nacional de Ciencia y Tecnología; Vetenskapsrådet, Grant/Award Number: 2016-00803; Ruth and Nils-Erik Stenbäck Foundation

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING INFORMATION

This work is supported by grants to JH from the Swedish Research Council, the Swedish Heart-Lung Foundation, Knut and Alice Wallenberg Foundation, Ruth and Nils-Erik Stenbäck Foundation, Konsul ThC Bergh's Foundation, and Ellen, Walter and Lennart Hesselman Foundation, to EM-E from Consejo Nacional de Ciencia y Tecnología (CONACYT) México.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.