

# Subcutaneous Prostaglandin E<sub>2</sub> Restrains Airway Mast Cell Activity in vivo and Reduces Lung Eosinophilia and Th<sub>2</sub> Cytokine Overproduction in House Dust Mite-Sensitive Mice

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## Key Words

Allergic asthma · Cytokines · Mast cells · Prostaglandin E<sub>2</sub>

## Abstract

**Background:** Prostaglandin (PG) E<sub>2</sub> is thought to exert protective effects in the lungs. Accordingly, aerosolized PGE<sub>2</sub> prevents the experimentally induced airway response to allergen challenge in asthmatics. In vitro evidence indicating that functional PGE<sub>2</sub> receptors (EP) are expressed on human mast cells and that PGE<sub>2</sub> can alter cytokine production suggests that these phenomena may be involved in its beneficial effect in asthma. However, in vivo evidence is scarce. **Methods:** We assessed the effects of exogenous PGE<sub>2</sub> and of the EP1/EP3 agonist sulprostone on the murine airway response to house dust mite (HDM) allergens, a model that accurately reproduces the spontaneous exposure of allergic asthma patients to aeroallergens. We also analyzed the in vivo impact of PGE<sub>2</sub> on production in the murine airway of mast cell protease (mMCP)-1, a specific marker of lung mast cell activity, and on local production of cytokines. **Results:** Exogenous PGE<sub>2</sub>, but not sulprostone, reduced eosinophilic infiltration in HDM-sensitized mice by half and led to a strong reduction in airway Th<sub>2</sub> cytokine expression. These anti-

inflammatory effects were accompanied in vivo by a substantial reduction in HDM-induced upregulation of airway mMCP-1. Neither PGE<sub>2</sub> nor sulprostone had any effect on airway hyperresponsiveness to methacholine. **Conclusions:** Our results indicate that the anti-inflammatory effect of PGE<sub>2</sub> can be reproduced in vivo in HDM-sensitized mice and suggest that this protective effect is dependent in vivo on inhibition of the allergen-triggered proinflammatory activity of bronchial mast cells. Finally, the effect of PGE<sub>2</sub> is linked to reduced upregulation of airway Th<sub>2</sub> cytokines.

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## Introduction

Current treatments for allergic asthma do not halt the underlying process and are sometimes even incapable of controlling the symptoms of the disease [1]. Clinical and experimental data suggest that prostaglandins (PG) could play a defensive role in the lungs [2, 3], and that a more detailed understanding of their underlying mechanisms might help uncover new therapeutic targets in asthma. Notably, PGE<sub>2</sub> aerosols administered to patients before airway challenge inhibit early and late asthmatic respons-

es to the allergen and, possibly, the accompanying bronchial hyperresponsiveness to methacholine [4, 5], a finding also described for nonallergic asthma [6]. It has also been reported that there is a decrease in airway eosinophil titers after allergen challenge in asthmatics who are locally exposed to PGE<sub>2</sub> [4, 7]. Although the PGE<sub>2</sub> anti-inflammatory effect would be expected to be the result of a selective attenuation of the Th<sub>2</sub> response, in vitro studies have largely suggested suppression of Th<sub>1</sub> cytokines [8, 9]. A PGE<sub>2</sub> effect on airway cytokines has never been reported in vivo in humans, but the PGE<sub>1</sub> analogue misoprostol has been shown to reduce lung IL-5 production in patients with mild asthma [10]. Accordingly, in ovalbumin (OVA)-sensitized rats, intratracheal PGE<sub>2</sub>-induced reduction of allergen-driven bronchospasm and eosinophilia is accompanied by diminished expression of Th<sub>2</sub> cytokines [11]. To our knowledge, such an anti-Th<sub>2</sub> effect has never been demonstrated in allergen-sensitized mice. Kunikata et al. [12] recently reported no effect of exogenous 16,16-dimethyl (dm)-PGE<sub>2</sub>, a modified PGE<sub>2</sub> molecule, on the airway reaction to OVA; however, using a similar model, De Campo and Henry [13] observed a PGE<sub>2</sub> anti-inflammatory effect. Prior to this, the only results from which a presumptive benefit of PGE<sub>2</sub> in sensitized mice could be inferred were based on indirect data arising from experiments in which cyclooxygenase (COX), and hence PGE<sub>2</sub>, was inhibited in OVA-sensitive mice [14, 15].

In an attempt to elucidate the mechanisms involved in PGE<sub>2</sub>-mediated protection, Hartert et al. [7] showed reduced concentrations of PGD<sub>2</sub> in bronchoalveolar lavage (BAL) fluid from patients with mild allergic asthma exposed to PGE<sub>2</sub>, suggesting that mast cells are primary or secondary targets. However, in that study, the levels of histamine and tryptase, a mast cell-specific mediator, were unaffected, despite in vitro evidence of functional PGE<sub>2</sub> receptors on the surface of mast cells [16–19]. The characterization of PGE<sub>2</sub> receptors and the development of agonists and antagonists [20] have led to a resurgence of interest in the role of PGE<sub>2</sub> in asthma. It remains unclear whether one specific receptor is involved in the development of asthma, but EP3 has recently been found to exert protective effects in OVA-sensitized mice [12], and there is some evidence that it is altered in human asthma [21]. The use of animal models of asthma to analyze the in vivo balance of the opposing actions of PGE<sub>2</sub> observed in inflammatory cells in vitro is of particular interest. The recent development of an accurate animal model involving sensitization with house dust mite (HDM) [22, 23] provides a potentially valuable tool with which to gain

insight into the mechanisms through which PGE<sub>2</sub> exerts its effects.

In this study, we addressed the in vivo effect of exogenously administered PGE<sub>2</sub> and of the EP1/EP3 agonist sulprostone on airway hyperresponsiveness (AHR), airway eosinophil recruitment, and Th<sub>2</sub> cytokine expression in HDM-sensitized mice. We also analyzed the role of lung mast cells in the presumptive beneficial effects of PGE<sub>2</sub> by evaluating airway expression of mouse mast cell protease (mMCP)-1 (a mucosal mast cell-specific protease).

## Material and Methods

### *Mice and Experimental Groups*

Eight-week-old female BALBc mice (Harlan, Spain) were housed under a 12-hour light-dark cycle. Six experimental groups were established. The first 3 groups contained nonsensitized (control) mice: group 1 contained untreated mice (n = 15) and groups 2 and 3 contained PGE<sub>2</sub>-treated (n = 15) and sulprostone-treated (n = 5) animals, respectively. The second 3 groups contained allergen-sensitized mice: group 4 included untreated animals (n = 21) and groups 5 and 6 contained PGE<sub>2</sub>-treated (n = 21) and sulprostone-treated (n = 11) animals, respectively. All animal procedures were approved by the Ethics Committee for Animal Research of the Universitat Autònoma de Barcelona.

### *Intranasal Sensitization to House Dust Mite Allergens*

Sensitization to HDM was induced according to the procedure established by Cates et al. [22]. Briefly, the mice were exposed to a purified HDM extract (Alk-Abelló, Madrid, Spain) with a low lipopolysaccharide (LPS) content (<0.5 EU/dose, measured using the Charles River Endosafe Limulus Amebocyte Assay; Charles River Laboratories, Wilmington, Mass., USA). The allergen was administered intranasally at a dose of 25 µg/mouse in a 10-µl volume on 10 consecutive days. Immediately before administration of HDM, light anesthesia was induced in a chamber filled with 4% halothane delivered over a period of 2 min in 100% oxygen and maintained for 2 additional minutes with 2.5% halothane. Nonsensitized (control) animals were manipulated identically except that they received intranasal saline instead of HDM extract.

### *Subcutaneous PGE<sub>2</sub> and Sulprostone Administration*

Both HDM-exposed and nonexposed mice were treated with either PGE<sub>2</sub> (0.5 mg/kg) or sulprostone (80 µg/kg), an EP3 agonist with a slight EP1 effect. Both EP receptor agonists were injected subcutaneously daily on the same days as HDM, but administration was continued up to day 12 (i.e. 2 days after the allergen was withdrawn). The prostanoid treatment was provided 1 h before exposure to HDM. PGE<sub>2</sub> was purchased from Cayman (Tallinn, Estonia; ref. 14010) and the PG solution was prepared daily in phosphate-buffered saline (PBS) from a stock solution dissolved in dimethyl sulfoxide (DMSO) and stored at –20°C. The final concentration of DMSO injected was 0.1%. Sulprostone (Cayman, ref. 14765) was also prepared daily in PBS, and the solu-

tion administered contained 0.05% DMSO. The untreated mice underwent the same procedure, except that they received subcutaneous vehicle (PBS containing 0.1% DMSO) instead of the EP agonist.

#### *Assessment of Airway Hyperresponsiveness*

Airway response to methacholine (Sigma-Aldrich, Madrid, Spain) was assessed by non-invasive whole-body plethysmography (WBP) (Buxco Europe Ltd., Winchester, UK) using an established procedure [24, 25] in treated and untreated mice 24 h after the last exposure to either saline or HDM, while animals were still receiving PGE<sub>2</sub> or sulprostone treatment. Briefly, conscious mice from all groups were placed in the controlled airflow chambers of the WBP device and were first exposed to nebulized PBS and then to increasing doses of nebulized methacholine (6.25, 12.5, 25, 50 and 100 mg/ml) at 7-min intervals (2 min of nebulization and a 5-min delay before delivery of the next concentration). The reaction of the airways to methacholine was averaged and expressed as an enhanced pause (Penh), a unitless measure of airway reactivity. Dose-response curves for each group were established and Penh values were compared by 2-way ANOVA with a post-hoc Bonferroni test.

#### *Assessment of Airway Inflammation*

Three methods were used to assess the effect of PGE<sub>2</sub> and sulprostone on HDM-induced airway inflammation. Forty-eight hours after the last administration of HDM, the animals were anesthetized with halothane and sacrificed by intracardiac puncture and blood collection. BAL was performed by slowly infusing 0.3 ml of PBS (2% fetal bovine serum) twice and recovering it by gentle aspiration 30 s after delivery. An aliquot of BAL cells was stained with Turk solution (0.01% crystal violet in 1% acetic acid) and analyzed in a Neubauer chamber. Total airway cellularity was determined by counting cells in BAL samples from all mice. The lungs were then removed and the left lung was perfused with formaldehyde, immersed in the fixative, and embedded in paraffin for subsequent histology study. The second method used to assess inflammation was the eosinophil count in the lung parenchyma. Paraffin-embedded lung sections (3  $\mu$ m) were stained with Congo red and a blinded observer counted the total number of stained eosinophils twice in 3 histological sections per animal. The tissue area was measured using MIP 45 Advanced image analysis software (Microm España) in order to express the number of eosinophils per mm<sup>2</sup>. Finally, a differential cell count was performed in BAL cytopspins from some of the samples of each experimental group. The pelleted BAL cells were adhered to a slide using a cytocentrifuge (600 rpm, 6 min) for Diff-Quick staining. Relative numbers of eosinophils, lymphocytes, macrophages, and neutrophils were then determined. Comparisons between groups were made using the t test.

#### *Protein Expression of Th<sub>2</sub> and Th<sub>1</sub> Cytokines in the Lung*

Local protein expression of Th<sub>2</sub> (IL-13, IL-5) and Th<sub>1</sub> (IFN- $\gamma$ ) cytokines was assessed in the lung parenchyma. After sacrifice, the right lung lobe was kept at -80°C for protein extraction using a lysis buffer containing a cocktail of protease inhibitors (Mini complete tablet; Roche Diagnostics, Barcelona, Spain). Without defrosting the sample, 250  $\mu$ l of lysis buffer containing protease inhibitors was added and the resulting solution was homogenized. After incubation for 20 min on ice, the homogenized sam-

ple was centrifuged at 17,530 RCF for 10 min at 4°C and the supernatants were collected and frozen at -30°C. The quantity of total protein was measured with a protein assay kit (Sigma-Aldrich, Madrid, Spain; ref. P 5656). BAL fluid was also centrifuged at 400 RCF for 5 min at 4°C and supernatants were collected and stored at -80°C for analysis. The levels of the Th<sub>1</sub> cytokine IFN- $\gamma$  and of the Th<sub>2</sub> cytokines IL-13 and IL-5 in the lung protein extracts were determined using sandwich ELISA (Biosource, Barcelona, Spain; ref. KMC4021, KMC0051, and KMC2221, respectively). Cytokine protein levels were compared between groups using the t test.

#### *Evaluation of Lung Mast Cell Activity*

Two approaches were used to determine the activity of lung mast cells: counting the number of mast cells in the airways and measuring a specific mast cell marker. The number of mast cells was counted in lung sections after staining with the metachromatic dye toluidine blue and was expressed as mast cells per cm<sup>2</sup>, according to a previously described method [26]. In addition, lung mast cell activity was assessed and compared (t test) by quantifying production of mMCP-1 in the airways. mMCP-1 was analyzed in both lung protein extracts and BAL fluid using a highly specific and sensitive sandwich ELISA (Moredun Scientific Ltd., Scotland, UK; ref. MS-RM3). In addition, correlations were studied between mMCP-1 levels and each of the following 4 measures: number of infiltrating eosinophils and expression of IL-13, IL-5, and IFN- $\gamma$ . Groups containing 33 or 45 mice in which the 2 measures under study were assessed simultaneously were pooled for this analysis. Graphpad Prism software was used to establish the significance of the correlations between mMCP-1 and each of the 4 variables, and to calculate the correlation coefficient ( $r^2$ ).

## **Results**

#### *Effect of Subcutaneous PGE<sub>2</sub> and Sulprostone on Airway Inflammation and Hyperresponsiveness*

Airway inflammation was present in mice sensitized to HDM in the form of bronchovascular inflammatory plaques containing predominantly eosinophils, although it was essentially absent in nonsensitized animals (fig. 1a). This finding was confirmed by analysis of total cell count in BAL fluid and, in some of the animals (n = 10 per group), by a differential count of BAL cell cytopspins stained with Diff-Quick. HDM-sensitized mice developed AHR compared with vehicle-exposed animals (ANOVA  $p < 0.0001$ ). No adverse effects of subcutaneous administration of PGE<sub>2</sub> or sulprostone were observed in terms of general appearance, respiratory function, or lung histology. Subcutaneous PGE<sub>2</sub> significantly inhibited HDM-induced recruitment of eosinophils to the airways (fig. 1a, 2,  $p < 0.001$ ). Eosinophil infiltration was reduced by around 50% in the HDM-sensitized mice receiving PGE<sub>2</sub> compared with the untreated animals ( $23.0 \pm 2.0$  eosinophils/mm<sup>2</sup> compared with  $11.9 \pm 1.9$  eo-

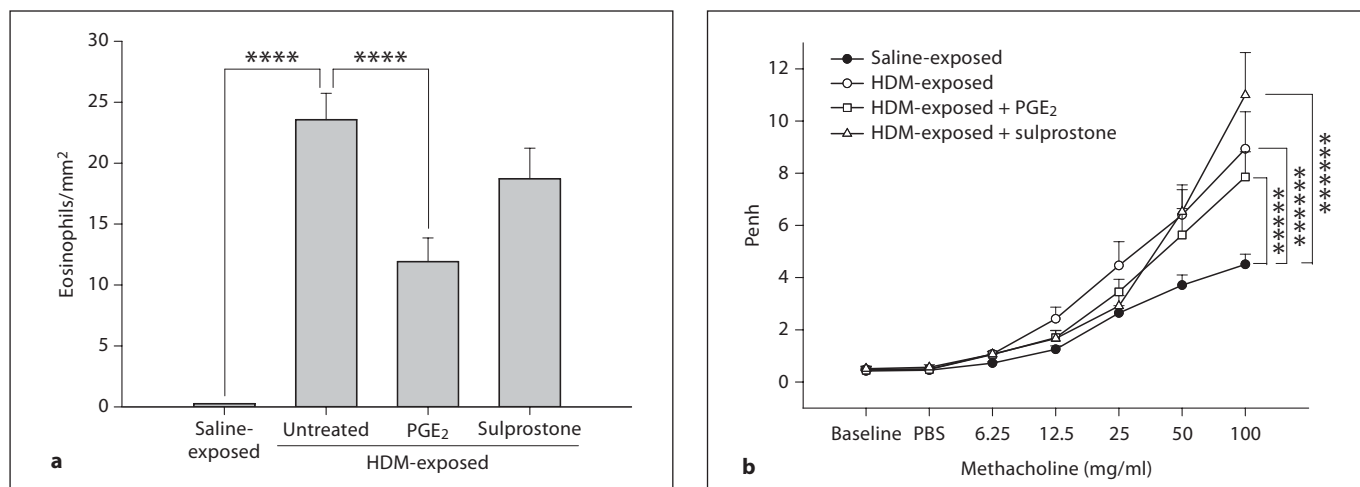


**Fig. 1.** Effect of exogenous PGE<sub>2</sub> and sulprostone on the airway response to house dust mite-induced asthma. **a** Number of eosinophils per mm<sup>2</sup> (eos/mm<sup>2</sup>) in lung tissue from animals treated with and without EP receptor agonists. Airway eosinophil infiltration was significantly lower in the presence of PGE<sub>2</sub> (n = 11), but remained unchanged with sulprostone (n = 11). **b** Airway hy-

perresponsiveness measured by whole body plethysmography in sensitive animals treated with or without EP receptor agonists. Neither PGE<sub>2</sub> (n = 21) nor sulprostone (n = 11) altered airway hyperresponsiveness to methacholine as shown by ANOVA (\*\*\*\* p < 0.001; \*\*\*\*\* p < 0.0005; \*\*\*\*\* p < 0.0001).

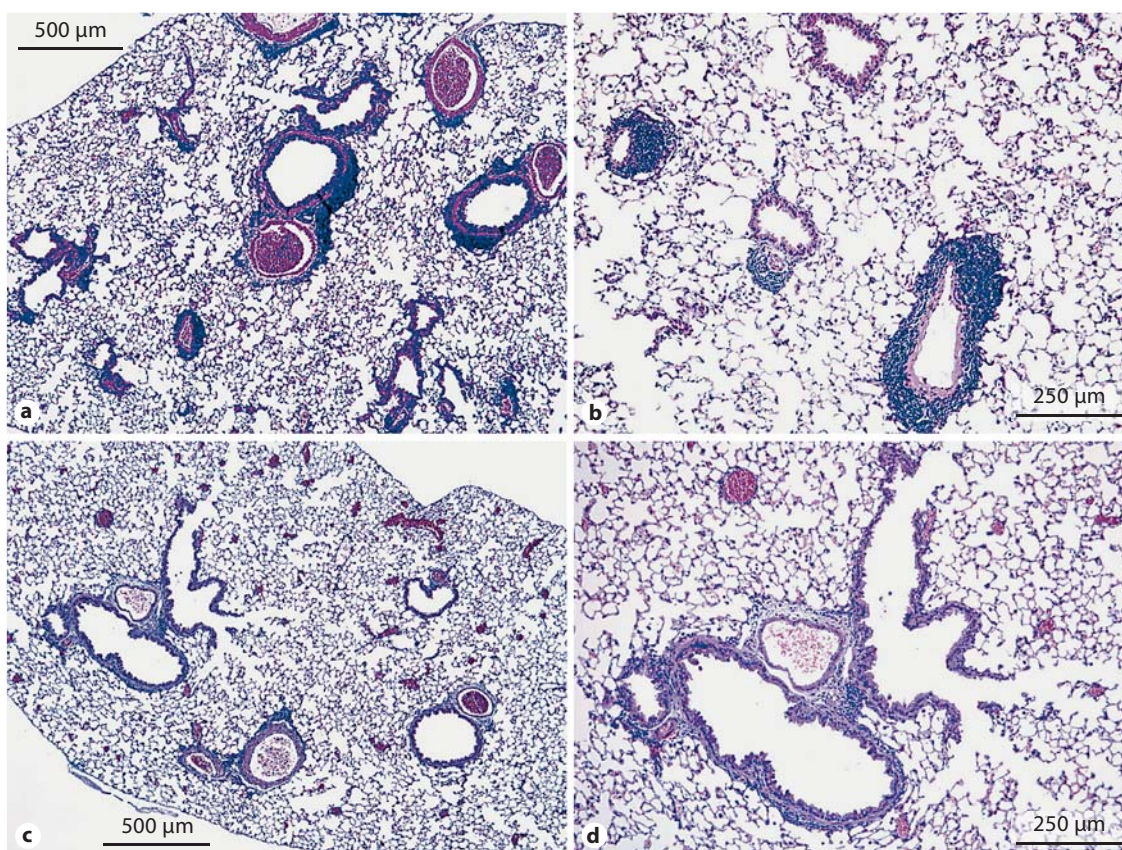


**Fig. 2.** Representative images at different scales of the level and type of bronchovascular eosinophilic infiltration in untreated (**a, b**) and PGE<sub>2</sub>-treated (**c, d**) house dust mite (HDM)-sensitive mice. Note the clearly visible effect of PGE<sub>2</sub> on the number and the size of the inflammatory foci.

sinophils/mm<sup>2</sup>; n = 11 in both groups). Total BAL cell counts also revealed a 50% decrease (p < 0.005) in the number of inflammatory cells in HDM-sensitized mice treated with PGE<sub>2</sub> (481,642 ± 62,340 cells/ml; n = 21) compared with untreated ones (829,190 ± 82,503 cells/ml; n = 21). Sulprostone did not affect airway inflammation, whether measured in lung sections (18.0 ± 2.0 eosinophils/mm<sup>2</sup>; n = 11), as shown in figure 1a, or in BAL cell counts (760,318 ± 102,501 cells/ml; n = 11). Methacholine-induced AHR was not affected by either PGE<sub>2</sub> or sulprostone (fig. 1b).

*Effect of Subcutaneous PGE<sub>2</sub> and Sulprostone on Lung Mast Cell Number and Activity*

To assess mast cell activity, production of the mucosal mast cell-specific mediator mMCP-1 was analyzed in lung parenchyma extracts (fig. 3) and in BAL fluid. Local expression of mMCP-1 protein was significantly higher in untreated HDM-exposed mice than in saline-exposed mice (25-fold increase, from 3,919 ± 1,039 pg/ml [n = 5] to 99,626 ± 15,442 pg/ml [n = 11]; p < 0.005). To ensure that the differences in mMCP-1 expression were not attributable to changes in mast cell number, we analyzed the number of parenchymal mast cells and the concentration of mMCP-1 in BAL fluid. As for metachromatic staining, similar numbers of airway mast cells (table 1) were observed in nonsensitized and untreated sensitized animals (7.8 ± 1.2 mast cells/cm<sup>2</sup> [n = 5] and 5.6 ± 0.9 mast cells/cm<sup>2</sup> [n = 11], respectively). The mMCP-1 protein concentration in BAL fluid displayed a similar pattern to that seen in the parenchyma, protease concentrations in the BAL fluid of HDM-exposed mice were higher than those in the controls (8,505 ± 1,199 pg/ml [n = 11] vs. 3,223 ± 287 pg/ml [n = 5]; p < 0.05). The observed increase in the activity of lung mast cells in HDM-sensitized mice was significantly inhibited by exogenous PGE<sub>2</sub>. Following administration of PGE<sub>2</sub>, the release of protease was reduced to less than a third of that seen in untreated HDM-exposed animals (99,626 ± 15,442 pg/ml [n = 11] vs. 30,980 ± 7,009 pg/ml [n = 11]; p < 0.001) (fig. 3). Similarly, mMCP-1 levels in BAL fluid were diminished in the presence of PGE<sub>2</sub> (8,505 ± 1,199 pg/ml in untreated sensitized animals [n = 11] compared with 4,949 ± 982 pg/ml in PGE<sub>2</sub>-treated ones [n = 11]; p < 0.05). No significant change was observed in the number of mast cells in the airway of mice treated with PGE<sub>2</sub> or sulprostone (table 1). Moreover, sulprostone had no significant effect on mMCP-1 production (90,485 ± 22,944 pg/ml in parenchymal extracts [n = 11] and 6,254 ± 515 pg/ml in BAL fluid [n = 11]).

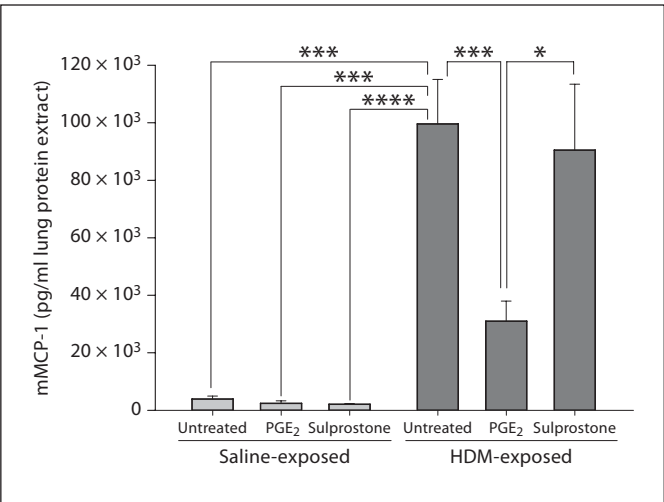


**Fig. 3.** Effect of exogenous PGE<sub>2</sub> or sulprostone on lung mast cell activity in a house dust mite (HDM)-induced murine model of asthma. The levels of mast cell protease-1 protein, a mucosal mast cell-specific mediator, were increased in the lungs of sensitized (n = 11) compared with nonsensitized (n = 5) mice. That increase was prevented by PGE<sub>2</sub> (n = 11) but not by sulprostone (n = 11) (\* p < 0.05; \*\*\* p < 0.005; \*\*\*\* p < 0.001).

**Table 1.** Number of airway mast cells in HDM-sensitized mice exposed to PGE<sub>2</sub> or sulprostone

|      | Non-sensitized mice |                          |                     | HDM-sensitized mice |                          |                     |
|------|---------------------|--------------------------|---------------------|---------------------|--------------------------|---------------------|
|      | non-treated         | treated PGE <sub>2</sub> | treated sulprostone | non-treated         | treated PGE <sub>2</sub> | treated sulprostone |
| Mean | 7.76                | 5.87                     | 5.13                | 5.57                | 4.53                     | 3.83                |
| SEM  | 1.17                | 0.96                     | 1.20                | 0.94                | 0.87                     | 0.57                |

Number of metachromatically stained mast cells infiltrating the airway of treated and non-treated mice (mast cells/cm<sup>2</sup>) exposed to house dust mite (HDM) or vehicle. No significant differences in mast cell count were detected between any of the studied conditions.

*Effect of Subcutaneous PGE<sub>2</sub> and Sulprostone on Th<sub>2</sub> and Th<sub>1</sub> Cytokines in vivo*

Expression of the Th<sub>2</sub> cytokines IL-13 and IL-5, but not of the Th<sub>1</sub> cytokine IFN-γ, was significantly higher in HDM-exposed mice than in saline-exposed mice (fig. 4). IL-13 increased 15-fold, from 60 ± 5 pg/ml (n = 5) to 913 ± 200 pg/ml (n = 11) (p < 0.05), and IL-5 dis-

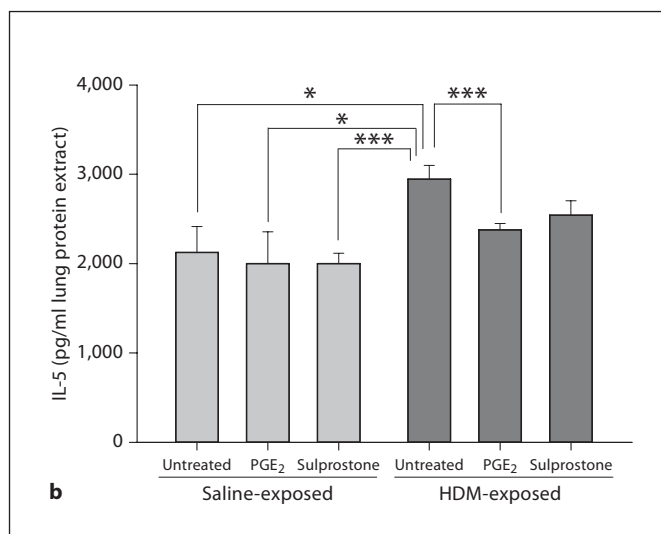
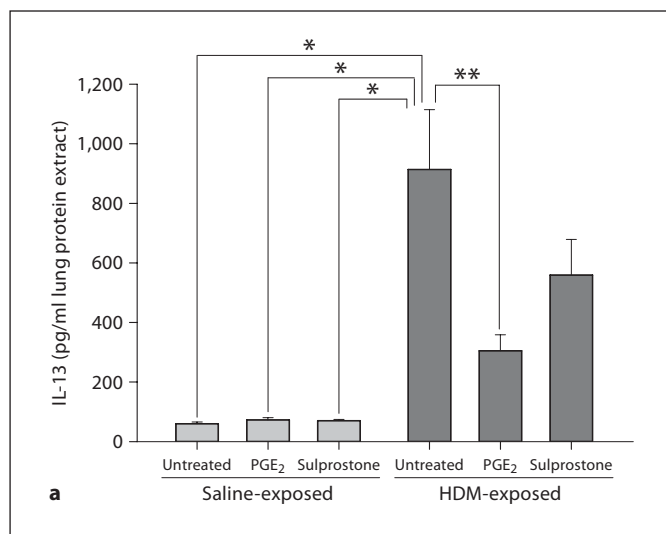
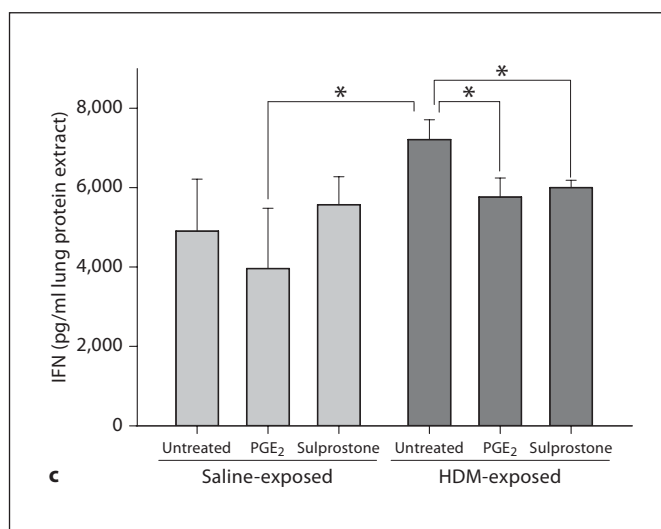


**Fig. 4.** Effect of PGE<sub>2</sub> and sulprostone on the production of Th<sub>2</sub> and Th<sub>1</sub> cytokines in house dust mite (HDM)-induced airway reactivity. **a** IL-13 was increased in the lungs of HDM-exposed mice (n = 11) compared with nonexposed animals (n = 5). PGE<sub>2</sub> (n = 11) reduced the increase to a third of the levels in untreated HDM-sensitized mice without affecting the baseline production of the cytokine. Sulprostone (n = 11) did not lead to significant changes in IL-13 levels. **b** Lung IL-5 was increased in sensitized mice; the increase was slightly inhibited by PGE<sub>2</sub> but not by sulprostone. While the Th<sub>1</sub> cytokine IFN-γ (**c**) was not significantly increased in the sensitive animals, its production was slightly reduced in response to both PGE<sub>2</sub> and sulprostone treatment (\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.005).



played a significant 1.4-fold increase from  $2,124 \pm 291$  pg/ml (n = 5) to  $2,945 \pm 154$  pg/ml (n = 11) (p < 0.05). PGE<sub>2</sub> significantly inhibited overproduction of Th<sub>2</sub> cytokines in the HDM-exposed airways but did not alter baseline cytokine production (control animals). Levels of IL-13 observed in animals receiving treatment were a third of those in the untreated sensitized ones ( $304 \pm 54$  pg/ml [n = 11] vs.  $913 \pm 200$  pg/ml [n = 11]; p < 0.01). IL-5 production was reduced from  $2,945 \pm 154$  pg/ml (n = 11) to  $2,378 \pm 70$  pg/ml (n = 11) in animals treated with PGE<sub>2</sub> (p < 0.005). The EP1/EP3 agonist sulprostone had no effect on overproduction of either of the Th<sub>2</sub> cytokines. A slight reduction in IFN-γ concentration was observed in response to treatment of the animals with either PGE<sub>2</sub> ( $7,210 \pm 497$  pg/ml [n = 11] in untreated animals

and  $5,760 \pm 483$  pg/ml [n = 11] in PGE<sub>2</sub>-treated HDM-sensitized mice; p < 0.05) or sulprostone ( $7,210 \pm 497$  pg/ml [n = 11] in untreated and  $6,000 \pm 186$  pg/ml [n = 11] in sulprostone-treated mice exposed to HDM; p < 0.05).

#### *Correlation between Mast Cell Activity and Markers of Inflammation in vivo*

To evaluate a potential statistical association between lung mast cell activity and fluctuations in the inflammatory process in HDM-sensitive mice, we analyzed the correlation between mMCP-1 levels and eosinophil count (pool of 33 mice from different experimental groups), and the correlation between mMCP-1 and the concentration of each individual cytokine (pool of 45 mice from differ-

ent groups). There was a statistically significant ( $p < 0.0001$ ) 48% correlation between mMCP-1 levels and eosinophil count. Similarly, there was a 61% correlation between lung mMCP-1 expression and lung IL-13 expression ( $p < 0.0001$ ) and a 21% correlation between mMCP-1 levels and lung IL-5 expression ( $p < 0.005$ ). No correlation was found between mMCP-1 and IFN- $\gamma$  expression.

## Discussion

We have shown that subcutaneously administered PGE<sub>2</sub> prevents allergen-induced airway eosinophilia and attenuates overproduction of Th<sub>2</sub> cytokines in HDM-sensitive mice. These PGE<sub>2</sub>-mediated anti-inflammatory effects are paralleled by a substantial inhibition of lung mast cell activity. The diminished bronchovascular inflammatory process observed was not accompanied by a change in AHR to methacholine. Finally, the EP1/EP3 agonist sulprostone was found to have no effect on lung inflammation or AHR.

Despite the reported protective effect of PGE<sub>2</sub> in human allergic asthma [4, 5], to our knowledge, no unequivocal data have been provided to indicate such an effect in mice [12, 13]. To address this and the scarcity of in vivo animal studies, we evaluated the impact of exogenous PGE<sub>2</sub> on allergen-triggered murine airway pathology. One of the main advantages of the approach lay in the model used, since the features of the recently developed HDM-sensitive mouse model (daily delivery exclusively through the airways of a natural aeroallergen known to cause human asthma) provide ideal conditions to mimic the human disease [27]. An intensive single-dose treatment protocol with PGE<sub>2</sub> was used with the purpose of ensuring that effective prostanoid levels would be present during the relevant phases of the process, regardless of the clinical relevance of such concentrations or the optimal dose-effect ratio. Nonetheless, it should be noted that the PGE<sub>2</sub> dose used in our study was lower than that shown to be effective in vivo in models of nonallergic conditions [28, 29], and that in our setting, neither PGE<sub>2</sub> nor sulprostone induced any observable adverse reactions. Exogenous PGE<sub>2</sub> reduced eosinophil infiltration in the airways of HDM-sensitized mice to half that seen in untreated animals. In contrast to our results, Kunikata et al. [12] did not find a dm-PGE<sub>2</sub>-induced effect on inflammatory cells infiltrating the airways, while in a similar OVA model, De Campo and Henry [13] did. This difference could be due to procedural issues. Irrespective of

the reasons underlying this discrepancy, in our view, the HDM-sensitive murine model provides ideal conditions in which to address the mechanisms underlying the anti-inflammatory effect of PGE<sub>2</sub> and to identify potential new pharmacological targets.

Building on ex vivo data in allergen-stimulated splenocytes [22], we have also demonstrated in vivo increased production of Th<sub>2</sub> cytokines in the lungs of HDM-sensitive mice. Overproduction of Th<sub>2</sub> cytokines was prevented by PGE<sub>2</sub>, and expression of IFN- $\gamma$  was slightly reduced, even though its production was not increased by HDM allergens. Although the effect of PGE<sub>2</sub> on cytokines was strong, it is noteworthy that IL-13 expression did not reach baseline values, whereas IL-5 may have done. Also noteworthy is the fact that baseline IL-5 levels were rather high, a consistent finding in our experience with this model. Although this did not prevent HDM from inducing an increase, it is worth analyzing further the causes of such lung IL-5 expression in nonsensitive mice, a finding also observed by others [30, 31]. It appears likely that the anti-eosinophilic potential of PGE<sub>2</sub> we observed in vivo will be mediated, at least in part, by IL-5 [32]. Accordingly, Martin et al. [11] found that intratracheal PGE<sub>2</sub> reduced both IL-5 mRNA expression and eosinophilia in a rat model of asthma induced by a combination of OVA and *Bordetella pertussis*. In conclusion, the reported effect of PGE<sub>2</sub> in reducing mainly Th<sub>1</sub> but also Th<sub>2</sub> inflammatory components in vitro [9, 16] was therefore confirmed in HDM-sensitive mice and now awaits confirmation in humans.

In our study, sulprostone did not affect airway inflammation or overexpression of Th<sub>2</sub> cytokines, suggesting that the EP3 receptor is not a major contributor to the observed anti-inflammatory effect of PGE<sub>2</sub>. This is somewhat surprising considering the findings of Kunikata et al. [12], although further experiments will help to elucidate whether these discrepancies have biological significance or are due to methodological differences such as the sensitizing allergen used or the genetic background of the animals.

Since PGE<sub>2</sub> has been reported to stabilize mast cells from human lungs and other tissues in vitro [16, 18], we assessed airway mast cell activity as an initial step in identifying the cells responsible for mediating its anti-inflammatory effects in vivo. Surprisingly, PGE<sub>2</sub>, but not sulprostone, led to a substantial decrease in allergen-induced mast cell activity in the lungs. This supports the hypothesis that PGE<sub>2</sub> exerts a restraining effect on mast cells [33] in vivo whether directly or through prior inter-

actions with other cell populations. We recently established the same hypothesis for endogenous PGE<sub>2</sub> in OVA-sensitive mice [34]. The actual impact on airway pathology of the modulation of mast cells by PGE<sub>2</sub> needs to be determined, as it could have effects that extend beyond the early stages of the disease [35]. From our experimental approach, we cannot infer whether the PGE<sub>2</sub> effect on the activity of mast cells is direct or rather a result of the prostanoid's effect on a mediating cell population. Nonetheless, given the reported presence of EP receptors on the surface of mast cells *in vitro*, one likely explanation in our view is that PGE<sub>2</sub> is acting, at least in part, directly on mast cells. Along those lines, the lack of a clear sulprostone effect and the reported presence of stabilizing EP2 receptors on the surface of mast cells [16, 18] suggest EP2 as a main candidate to mediate the observed PGE<sub>2</sub>-restraining effect on mast cells. This is not totally surprising, since EP3 stimulation is often viewed as a pro-inflammatory and pro-allergic strategy [17, 19]. Our observed PGE<sub>2</sub>-induced blockade of mast cell activity could explain the reduced levels of PGD<sub>2</sub> in the airways of asthmatics reported by Hartert et al. [7], since this mediator is primarily attributed to mast cells, although these authors did not observe parallel changes in the levels of either the human mast cell-specific mediator tryptase or histamine. Inhibition of PGD<sub>2</sub> production would at least partly account for the observed effect of PGE<sub>2</sub> on airway eosinophilia [36]. Regardless of the mast cell mediator that induces or contributes to airway inflammation in HDM-sensitive mice, there was a correlation between mMCP-1 concentrations and the eosinophil count, indicating that fluctuations in mast cell activity were associated with changes in eosinophil recruitment, and therefore suggesting that the observed reduction in eosinophilic infiltration in response to PGE<sub>2</sub> treatment might be a result of the diminished mast cell activity [37]. The difficulties encountered by Hartert et al. [7] in demonstrating a mast cell-specific role in the detected PGE<sub>2</sub> effect on eosinophil infiltration led us to analyze the levels of mMCP-1 in both BAL fluid and lung extracts. Having taken an approach in which the observations were confirmed using different techniques, together with the absence of alterations in mast cell counts in the lung, we can conclude that the observed increase in mMCP-1 protein in the lung parenchyma of untreated animals was the result of increased mast cell activity in HDM-sensitized mice rather than an increase in mast cell numbers. Similarly, the PGE<sub>2</sub>-induced reduction of mMCP-1 expression is presumably due primarily to inhibition of mast cell activation rather than to a reduction in the number of mast

cells. It is noteworthy that mice treated with PGE<sub>2</sub> or sulprostone, whether sensitive to HDM or not, have a lower number of airway mast cells than saline-treated animals, although this is not statistically significant. Although the influence of such figures in the expression of mMCP-1 in mast cells seems irrelevant, one could speculate that prostanoids might preclude mast cell precursors from homing to tissues. We are unaware of any report addressing this issue, which is beyond the scope of our experiment, although it does warrant further investigation.

We also found a correlation between mast cell activity and both IL-13 and IL-5 levels. Again, this prompts us to hypothesize that the reduction in cytokine concentrations driven by PGE<sub>2</sub> could be linked to reduced mast cell activity. Although the two Th<sub>2</sub> cytokines seem to be produced mainly by T cells, airway mast cells cannot be completely ruled out as a partial source since Feng et al. [16] described an effect of PGE<sub>2</sub> on the *in vitro* production of IL-5 by human mast cells, and it has been demonstrated that both IL-5 and IL-13 are produced by mouse lung mast cells [35], and up-regulated in the airway mast cells of asthmatic patients [38, 39].

Neither PGE<sub>2</sub> nor sulprostone had an effect on AHR, despite the fluctuating levels of IL-13. The sensitizing allergen (HDM or OVA) or the experimental approach could account for this discrepancy with previous observations [40]. Whatever the cause, in the HDM model, mediators other than IL-13 are responsible for altered airway smooth muscle reactivity, a possibility to be considered in the light of previous findings [41]. Our data also question whether there is a separation between the mechanisms leading to AHR and inflammation, or rather if there is a need to achieve a higher anti-inflammatory threshold in order to modify airway reactivity. Dissociation of airway inflammatory and bronchial smooth muscle responses has been reported previously in asthmatics [42], implying that the pathophysiologic relationship between airway inflammation and AHR is not as simple as initially suggested. Finally, in our study, inhibition of mast cell activity had no effect on AHR. A number of explanations could account for this apparent discrepancy, namely, differences in the asthma model, insufficient reduction in mast cell activity, selective inhibition of mast cell mediators by PGE<sub>2</sub>, or differential mast cell involvement in acute and chronic allergen-induced AHR [26].

In summary, to our knowledge this is the first report showing an anti-inflammatory effect of PGE<sub>2</sub> in a HDM-induced mouse model of asthma. Our findings suggest for the first time that this beneficial effect of PGE<sub>2</sub> in al-

lergic asthma may be mediated by a restraining effect on airway mast cells. We hope that our data will help consolidate the HDM-induced asthma model as a valuable system in which to further investigate the mechanisms of PGE<sub>2</sub> protection and identify new therapeutic targets in allergic asthma.

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