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Locally administered prostaglandin E2 prevents aeroallergen-induced airway sensitization in mice through immunomodulatory mechanisms

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ABSTRACT

Prostaglandin E2 attenuates airway pathology in asthmatic patients and exerts a protective effect in antigen-sensitized mice when administered systemically. We aimed to establish the consequences of intranasal PGE2 administration on airway reactivity to aeroallergens in mice and reveal the underlying immunoinflammatory mechanisms.

PGE2 was administered either daily during a 10-day exposure to house dust mite (HDM) extracts or for limited intervals. Airway hyperreactivity was measured by whole-body and invasive plethysmography. The phenotypes of lung immune cells and cytokine production were analysed by flow cytometry and ELISA, respectively.

Airway hyperreactivity was sustainably reduced only when PGE2 administration was restricted to the initial 5 days of exposure to HDM. Lung inflammation, IL-4 production, and airway mast cell activity were also prevented under this early short-term treatment with PGE2. Interestingly, a Th2 response was already committed on day 5 of exposure to HDM. This was paralleled by GM-CSF and osteopontin upregulation and a decreased number of plasmacytoid dendritic and T regulatory cells, as well as a trend towards reduced IL-10 expression. Local PGE2 administration prevented the increase of airway IL-13 and osteopontin and kept lung plasmacytoid dendritic cell counts close to baseline. GM-CSF and Tregs were unaffected by the treatment.

These findings suggest that the protection provided by PGE2 is a result of the modulation of early lung immunomodulatory mechanisms, and possibly a shift in the balance of dendritic cells towards a tolerogenic profile.

1. Introduction

Current treatments of allergic asthma do not halt the underlying process and are often unable to control the symptoms of the disease. The fact that endogenous-like compounds, such as synthetic corticosteroids, remain the first-line treatment against asthma sets solid grounds for exploring natural protective pathways as an efficient strategy in search for new pharmacological targets. Local administration of prostaglandin (PG) E2, an endogenous molecule, to asthmatic patients challenged experimentally with allergens resulted in improved airway function and reduced inflammation [1–3]. In rodent models, we and others have shown that PGE2 alleviates ovalbumin (OVA)-driven inflammation, whereas COX-2 blockade, which leads to reduced internal PGE2 production, increases airway reactivity [4–7]. We have recently reached similar conclusions using aeroallergen-sensitized mice [8,9]. Notably, we demonstrated that mice exposed to house dust mite (HDM) extracts were protected from airway hyperreactivity and inflammation when given subcutaneous PGE2. There is therefore substantial evidence highlighting a beneficial effect of PGE2 in the airways *in vivo*; however, little is known about the underlying mechanisms.

Abbreviations: PGE2, prostaglandin E2; HDM, house dust mite; pDC, plasmacytoid DC; mDC, myeloid DC; OPN, osteopontin; AHR, airway hyperresponsiveness; MC, mast cell; mMCP-1, mouse mast cell protease-1; WBP, whole body plethysmography.

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It has been proposed that PGE2 exerts anti-inflammatory and immunomodulatory effects both *in vitro* and *in vivo*. For instance, PGE2 has been shown to regulate migration and differentiation of T-cell subsets, inhibit Th2 cytokine production [10], upregulate the immunosuppressive cytokine IL-10 [11], and modulate IgE expression [12] and mast cell activity [8,13]. The ability of PGE2 to modulate dendritic cell (DC) behaviour *in vitro*, either through inhibition [14,15] or activation [16] of this cell population, has also been reported. This finding is potentially relevant to asthma, because the balance between plasmacytoid and myeloid dendritic cells (pDCs and mDCs, respectively) in the lungs may shift from sensitization towards tolerance of aeroallergens [17]. The cytokines GM-CSF and osteopontin (OPN) may influence this DC ratio. GM-CSF is a pro-inflammatory molecule shown to be essential for the development and maintenance of aeroallergen-driven Th2 responses in HDM-sensitized mice through DC maturation and activation [18,19]. OPN, in turn, is a pro-inflammatory cytokine recently shown to be upregulated in asthmatic patients [20,21] and in murine models of asthma, where it induces airway inflammation and airway hyperresponsiveness (AHR) and recruits DC subsets to the airways [22]. Despite the relevance of these mediators, no studies have addressed the effect of PGE2 on GM-CSF or OPN. In addition to pDCs, regulatory T cells (Tregs) suppress Th2 responses by secreting IL-10 and TGF-β. These immunosuppressive cells are known to downregulate Th2 cytokines and prevent IgE production, mast cell activation, and DC maturation [23]. In patients with asthma, there are fewer Tregs, and those present are functionally impaired [24]. In contrast, there are more Tregs in patients effectively treated with immunotherapy [25]. To date, to our knowledge, no studies have addressed the impact of PGE2 on Tregs in asthma.

A detailed understanding of the effect of PGE2 on these cellular and molecular immunoregulatory and proinflammatory events may provide clues to the internal mechanisms by which PGE2 exerts its protective effect in allergic asthma. This, in turn, might contribute to the identification of crucial molecules or processes to be tested as targets for anti-asthma drugs.

To help understand the protective nature of PGE2 in allergic asthma, we first studied the effect of intranasal PGE2 in mice sensitized to HDM aeroallergens. We then explored the relevance of different PGE2 treatment intervals to help narrow the range of candidate airways molecules involved in this effect. Lastly, we analysed the effect of PGE2 on specific early lung immunological and inflammatory events and on crucial immune mediators.

2. Materials and methods

2.1. Mice

Female BALB/c mice (8–9 weeks old, Harlan Iberica, Spain) were kept under a 12-h light–dark cycle with *ad libitum* access to food and water. All experiments were approved by the Ethics Committee for Animal Research of the Universitat Autònoma de Barcelona (Spain).

2.2. Sensitization to HDM aeroallergens

Mice were exposed to a purified HDM extract (provided by Alk-Abelló, Spain) with a low lipopolysaccharide content (<0.5 EU/dose, using the Endosafe LAL Assay, Charles River Laboratories, Wilmington, MA, USA). The HDM extract was administered daily intranasally (i.n.) at a dose of 25 μg/mouse in a volume of 35 μL for either 5 or 10 consecutive days under isoflurane-induced anaesthesia. Non-sensitized (control) animals were manipulated identically except that they received i.n. saline instead of the HDM extract. Airway reactions to aeroallergens were evaluated after the 10-day exposure protocol. Alternatively, some responses were assessed after 5 days of exposure to the extract (for assessment of the early immunological events).

2.3. Intranasal PGE2 treatments in the 5- and 10-day HDM exposure protocols

PGE2 (Cayman ref. 14010) was prepared daily in PBS from a 100 mg/mL stock solution dissolved in DMSO and stored at −20 °C. Thirty-five microliters of PGE2 solution were administered i.n. (0.3 mg/kg) 1 h before each HDM exposure. The optimal dose was selected on the basis of previous studies [8,26]. Untreated mice underwent the same manipulation, except that they received vehicle alone (PBS in 0.1% DMSO) instead of the PGE2 solution.

Mice exposed to HDM for 10 consecutive days (day 0 to day 9) were subject to four different PGE2 treatment time courses (Fig. 1). Such treatment intervals were established to narrow the field of relevant phases, or molecules, targeted by PGE2. Hence, time courses were selected to examine potential PGE2 effects on early immunological events and late effector mechanisms:

- *Treatment schedule* (*a*): PGE2 was administered i.n. daily during the entire period of exposure to HDM, *i.e.*, PGE2 was instilled into the nostrils of the mice once a day from day −1 (1 day before starting HDM sensitization) until day 11.
- *Treatment schedule* (*b*): PGE2 was delivered i.n. on 2 different days during exposure to HDM (days 4 and 9), *i.e.*, early and late instillation of PGE2.
- *Treatment schedule* (*c*): PGE2 was administered i.n. for the last 5 days (days 7–11).
- *Treatment schedule* (*d*): PGE2 was administered i.n. for the initial 6 days (days −1 to 4).

For each one of the treatment schedules described above, four experimental conditions were tested (mice were either HDM-sensitized or not and either PGE2-treated or not).

In all four PGE2 administration schedules, the airway response was assessed on day 11, *i.e.*, 48 h after the last HDM challenge, except for the measurement of airway reactivity that was undertaken 24 h after the last challenge.

For some studies, airway immunoinflammatory responses were assessed after 5 days of exposure to HDM extract (from days 0 to 4) during which the mice were also given PGE2.

2.4. Effect of intranasal PGE2 on airway reactivity after 10-day HDM exposure

2.4.1. Assessment of airway responsiveness

Airway reactivity was analysed in all animals 24 h after the last exposure to HDM using either unrestrained whole-body plethysmography (WBP; Buxco Europe, Winchester, UK) as previously described [27] or an invasive technique to measure airway resistance with two Finepointe Series RC sites (Buxco Europe). As for the latter method, in brief, mice were anaesthetized with an i.p. injection of ketamine/xylazine. The trachea was exposed and cannulated. The mice were mechanically ventilated at 120 breaths/min and a tidal volume of 12.5 ml/Kg. Baseline readings for resistance were recorded, and increasing doses of aerosolised methacholine were administered. The average of the maximum response for each dose was then calculated.

2.4.2. Quantification of airway inflammation

Animals were sacrificed 48 h after the last HDM administration. Bronchoalveolar lavage (BAL) was performed slowly by infusing 0.3 mL PBS (2% foetal bovine serum) twice and recovering it by gentle aspiration after 30 s. An aliquot of the BAL cells was stained with