

Prostaglandin E₂ suppresses allergic sensitization and lung inflammation by targeting the E prostanoid 2 receptor on T cells

Zbigniew Zaslona, PhD,^a Katsuhide Okunishi, MD, PhD,^a Emilie Bourdonnay, PhD,^a Racquel Domingo-Gonzalez, BS,^a Bethany B. Moore, PhD,^a Nicholas W. Lukacs, PhD,^b David M. Aronoff, MD,^c and Marc Peters-Golden, MD^a *Ann Arbor, Mich*

Background: Endogenous prostanoids have been suggested to modulate sensitization during experimental allergic asthma, but the specific role of prostaglandin (PG) E₂ or of specific E prostanoid (EP) receptors is not known.

Objective: Here we tested the role of EP2 signaling in allergic asthma.

Methods: Wild-type (WT) and EP2^{-/-} mice were subjected to ovalbumin sensitization and acute airway challenge. The PGE₂ analog misoprostol was administered during sensitization in both genotypes. *In vitro* culture of splenocytes and flow-sorted dendritic cells and T cells defined the mechanism by which EP2 exerted its protective effect. Adoptive transfer of WT and EP2^{-/-} CD4 T cells was used to validate the importance of EP2 expression on T cells.

Results: Compared with WT mice, EP2^{-/-} mice had exaggerated airway inflammation in this model. Splenocytes and lung lymph node cells from sensitized EP2^{-/-} mice produced more IL-13 than did WT cells, suggesting increased sensitization. In WT but not EP2^{-/-} mice, subcutaneous administration of misoprostol during sensitization inhibited allergic inflammation. PGE₂ decreased cytokine production and inhibited signal transducer and activator of transcription 6 phosphorylation by CD3/CD28-stimulated CD4⁺ T cells. Coculture of flow cytometry-sorted splenic CD4⁺ T cells and CD11c⁺ dendritic cells from WT or EP2^{-/-} mice suggested that the increased IL-13 production in EP2^{-/-} mice was due to the lack of EP2 specifically on T cells.

Adoptive transfer of CD4⁺ EP2^{-/-} T cells caused greater cytokine production in the lungs of WT mice than did transfer of WT CD4⁺ T cells.

Conclusion: We conclude that the PGE₂-EP2 axis is an important endogenous brake on allergic airway inflammation and primarily targets T cells and that its agonism represents a potential novel therapeutic approach to asthma. (*J Allergy Clin Immunol* 2013;■■■:■■■-■■■.)

Key words: Asthma, allergic sensitization, prostaglandin E₂, CD4 T cells

A link between prostanoids, lipid mediators derived from the COX metabolism of arachidonic acid, and asthma has long been appreciated. Approximately 10% of asthmatic patients have acute bronchoconstriction after taking aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit COX.¹ Furthermore, COX inhibition by NSAIDs and deletion of genes encoding COX isoenzymes have both been found to increase allergic inflammation in mouse models of asthma.²⁻⁵ Although these data strongly suggest the presence of an endogenous suppressive COX metabolite, the removal of which unmasks allergic inflammation, its precise identity remains uncertain. COX metabolites include 5 major bioactive prostanoids, prostaglandin (PG) E₂, prostacyclin, PGD₂, PGF_{2α}, and thromboxane A₂, and all of these have been implicated in various aspects of asthma pathogenesis.⁶⁻¹⁰

PGE₂ is the most abundant prostanoid of most tissues, and it can act on virtually all cell types. However, its effects on inflammatory and immune events are widely pleiotropic, in part reflecting its ability to ligate any of 4 G protein-coupled receptors, termed E prostanoid (EP) receptors 1 to 4, which activate distinct intracellular signaling programs. EP2 and EP4 signal by stimulating adenylyl cyclase to generate the second messenger cyclic AMP (cAMP), which is typically suppressive of inflammatory cell functions. By contrast, EP3, by inhibiting adenylyl cyclase, and EP1, by increasing intracellular Ca²⁺, typically activate leukocyte functions.¹¹ EP2 ligation by PGE₂ is a strong candidate mechanism to account for the suppressive effects of an endogenous prostanoid on allergic inflammation. First, EP2 has been shown to mediate many of the inhibitory actions of PGE₂ on various functions of immune cells,¹²⁻¹⁴ including T cells.^{15,16} Second, a polymorphism in the EP2 gene was reported to be associated with aspirin-induced asthma.¹⁷

Investigating the process of sensitization to allergen provides a window onto the generation of an adaptive immune response, a process that is integral to allergic asthma but clinically silent. PGE₂ has been implicated in the generation of T_H2 immune responses.¹⁸ On the other hand, its ability to inhibit T-cell

From the Department of Internal Medicine, Divisions of ^aPulmonary and Critical Care Medicine and ^cInfectious Disease, and ^bthe Department of Pathology, University of Michigan Medical School.

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Corresponding author: Marc Peters-Golden, MD, 6301 MSRB III, 1150 W Medical Center Dr, Ann Arbor, MI 48109-5642. E-mail: petersm@umich.edu. 0091-6749/\$36.00

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Abbreviations used

BALF: Bronchoalveolar lavage fluid
 cAMP: Cyclic AMP
 DC: Dendritic cell
 DMSO: Dimethyl sulfoxide
 EP: E prostanoïd
 Epac: Guanine nucleotide exchange protein directly activated by cAMP
 NSAID: Nonsteroidal anti-inflammatory drug
 OVA: Ovalbumin
 PG: Prostaglandin
 PKA: Protein kinase A
 STAT: Signal transducer and activator of transcription
 WT: Wild-type

proliferation¹⁹ and MHC class II expression^{20,21} also endows PGE₂ with the potential to interfere with adaptive immunity. In this study we sought to specifically interrogate the role of the PGE₂-EP2 axis in controlling sensitization and T_H2 polarization in a murine model of allergic asthma. Our data support an important suppressive role for endogenous PGE₂-EP2 signaling in allergic asthma and further identify EP2 on CD4⁺ T cells as the critical target mediating PGE₂-induced suppression of sensitization.

METHODS**Animals**

Mice harboring a targeted deletion of both alleles of the *ptger2* gene encoding the EP2 receptor were originally generated by Dr Richard Breyer (Vanderbilt University)²² and bred in the University of Michigan Unit for Laboratory Animal Medicine. Eight- to 12-week-old male mice were used for all experiments. Animals were treated according to National Institutes of Health guidelines for the use of experimental animals, with the approval of the University of Michigan Committee for the Use and Care of Animals.

Reagents

Albumin from chicken egg (ovalbumin [OVA]) was obtained from Sigma-Aldrich (St Louis, Mo). EP2 agonist (butaprost free acid), PGE₂, and PGE₂ stable analog (misoprostol) were obtained from Cayman Chemicals (Ann Arbor, Mich). EP4 agonist (Ono-AE1-329) and EP3 agonist (Ono-AE-248) were kind gifts from Ono Pharmaceuticals (Osaka, Japan). Dimethyl sulfoxide (DMSO) served as the vehicle control for PGE₂ and EP agonists. The protein kinase A (PKA)-specific cAMP analog 6-Bnz-cAMP (N6-benzoyladenosine-3',5'-cyclic monophosphate) and the guanine nucleotide exchange protein directly activated by cAMP (Epac) 1-specific cAMP analog 8-pCPT-2'-O-Me-cAMP (8-4-chlorophenylthio-2'-O-methyladenosine-3',5'-cyclic monophosphate) were obtained from the Biolog Life Science Institute (Howard, Calif).

Purification and culture of mouse splenic CD4⁺ T cells and CD11c⁺ dendritic cells

For coculture of splenic T cells and dendritic cells (DCs), a single-cell suspension of splenocytes from OVA-sensitized mice was washed with PBS/2 mmol/L EDTA/0.5% FCS, and Fc receptor-mediated and nonspecific antibody binding was blocked by addition of FcR Blocking Reagent (Miltenyi Biotec, Auburn, Calif). Cells were incubated with magnetic bead-conjugated anti-CD11c antibodies (Miltenyi Biotec), followed by magnetic separation, according to the manufacturer's instructions. Subsequently, the cell population enriched in CD11c⁺ cells was stained with CD11c antibody, and the cell population negative for CD11c cells was stained with CD4 antibody; both populations were flow sorted to high purity (>96%). Greater than 93% of

the CD4⁺ cell population that we isolated from the spleens of wild-type (WT) or EP2^{-/-} mice were CD3⁺ T cells. After sorting, purified CD11c⁺ DCs and CD4⁺ T cells were cocultured with 100 µg/mL OVA at a ratio of 1:10 in U-bottom 96-well plates for 3 days, and supernatant was collected for cytokine analysis by means of ELISA.

In vitro expansion and activation of CD4⁺ T cells

Ninety-six-well plates were coated with 0.5 µg/mL CD3 (BioLegend, San Diego, Calif) and 0.5 µg/mL CD28 (BD Bioscience, San Jose, Calif) antibodies for 4 hours at 37°C and washed with PBS to removed unbound soluble antibodies. Splenic CD4⁺ T cells were plated at a concentration of 2 × 10⁵ per well and cultured for 3 days.

OVA-induced asthma protocol and sample harvest

As previously described, asthma was induced by means of intraperitoneal injection of 20 µg of OVA (Sigma) mixed with 2 mg of alum (Thermo Scientific, Waltham, Mass),²³ followed 7 days later by 2 airway challenges with 1% OVA, unless indicated otherwise. This well-established protocol is known to result in eosinophilic inflammation and induction of T_H2 cytokines in bronchoalveolar lavage fluid (BALF). Samples were collected 24 hours after the last airway challenge. Total cells were counted, followed by differential counting of Wright-Giemsa-stained cytospin preparations. Lavage fluid recovered from the first 0.6-mL aliquot injected into the lung was analyzed by means of ELISA to assess cytokines. For IgE measurements, blood was collected from the inferior vena cava, and serum was isolated by means of centrifugation; after 1:1000 dilution, serum was analyzed for IgE by using ELISA.

In vivo misoprostol treatment

Misoprostol was administered *in vivo* according to a previously established protocol.¹⁴ To determine the effects of a stable PGE₂ analog on asthma, mice were injected subcutaneously with 200 µL of saline containing 50 µg of misoprostol in 0.5% DMSO 2 hours before and 10 hours after intraperitoneal sensitization or airway challenge with OVA; control mice received 200 µL of saline containing 0.5% DMSO alone.

CD4⁺ T-cell transfer

CD4⁺ T cells were isolated from spleens of WT or EP2^{-/-} OVA-sensitized mice after airway challenge by using magnetic beads for CD4⁺ selection (Miltenyi Biotec). Isolation was performed according to the manufacturer's instructions, and the purity of CD4⁺ T cells was greater than 90%. Five million CD4⁺ T cells were injected intravenously, and 24 hours later, mice were challenged with 3% OVA for 3 consecutive days; 24 hours after the last airway challenge, mice were killed, and samples were collected.

Data analysis

All data are displayed as means ± SEMs from 3 to 6 independent experiments, each using a different mouse, unless indicated otherwise. Statistical differences among treatment groups were estimated by using ANOVA with the Tukey *post hoc* test for multiple comparisons or by using the Student *t* test, as appropriate, with GraphPad Prism 5.0 software (GraphPad Software, San Diego, Calif). A *P* value of less than .05 was considered statistically significant.

RESULTS**Enhanced allergic inflammation in EP2^{-/-} animals**

PGE₂-EP2 signaling has been shown to be immunosuppressive in many different cell types and *in vivo* models.^{12,15} Here we used EP2-deficient (EP2^{-/-}) and EP2-sufficient (WT) mice to assess the importance of endogenous PGE₂-EP2 signaling in a model of OVA-induced allergic asthma.²⁴ After sensitization and

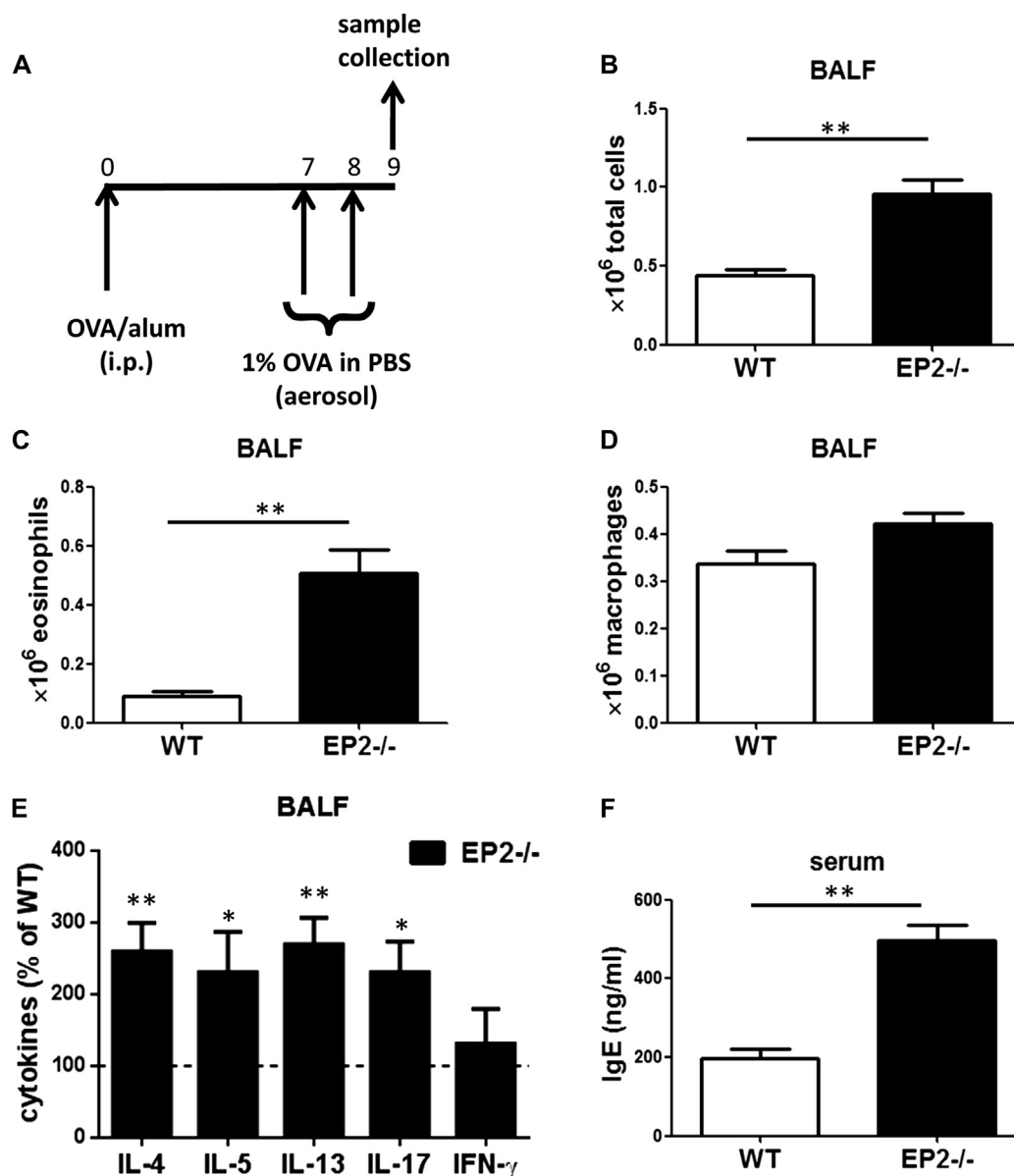


FIG 1. Enhanced allergic inflammation in EP2^{-/-} animals. **A**, Schematic representation of the experimental protocol. **B-D**, EP2^{-/-} mice have increased numbers of eosinophils and total cells in BALF after airway challenge. **E**, EP2^{-/-} mice have increased cytokine levels in BALF after airway challenge ($n = 4$). **F**, EP2^{-/-} mice have increased serum IgE levels. Samples were collected 24 hours after the second airway challenge ($n = 3$). * $P < .05$ and ** $P < .01$.

subsequent airway challenge using the protocol outlined in Fig 1, A, differential cell counts from BALF cytospin preparations revealed higher numbers of both total leukocytes and eosinophils in the airways of EP2^{-/-} mice (Fig 1, B-D). Additionally, mice deficient in EP2 exhibited increased BALF levels of the TH2 cytokines IL-4, IL-5, and IL-13, as well as IL-17, without any difference in levels of the TH1 cytokine IFN- γ (Fig 1, E). Except for IL-5, which was present at very low levels (approximately 20 pg/mL) and did not differ between the 2 genotypes, none of these other cytokines were detectable in serum. Lymphocytes and granulocytes each comprised less than 5% of the total cells found in the BALF, and there were no differences in their relative numbers between the 2 genotypes (data not shown). Consistent

with an increased systemic TH2 immune response, mice lacking EP2 also manifested higher serum IgE levels (Fig 1, F).

Augmented TH2 responses in EP2^{-/-} mice are present during sensitization

Lung lymph node cells and splenocytes were isolated from mice 24 hours after the second airway challenge and cultured for 3 days with varying concentrations of OVA. Cells of both types from EP2^{-/-} mice exhibited dose-dependent IL-13 production, which was greater than that from WT cells (Fig 2, A and B). IFN- γ production was likewise upregulated in splenocytes from EP2^{-/-} mice (data not shown), suggesting that enhanced immune

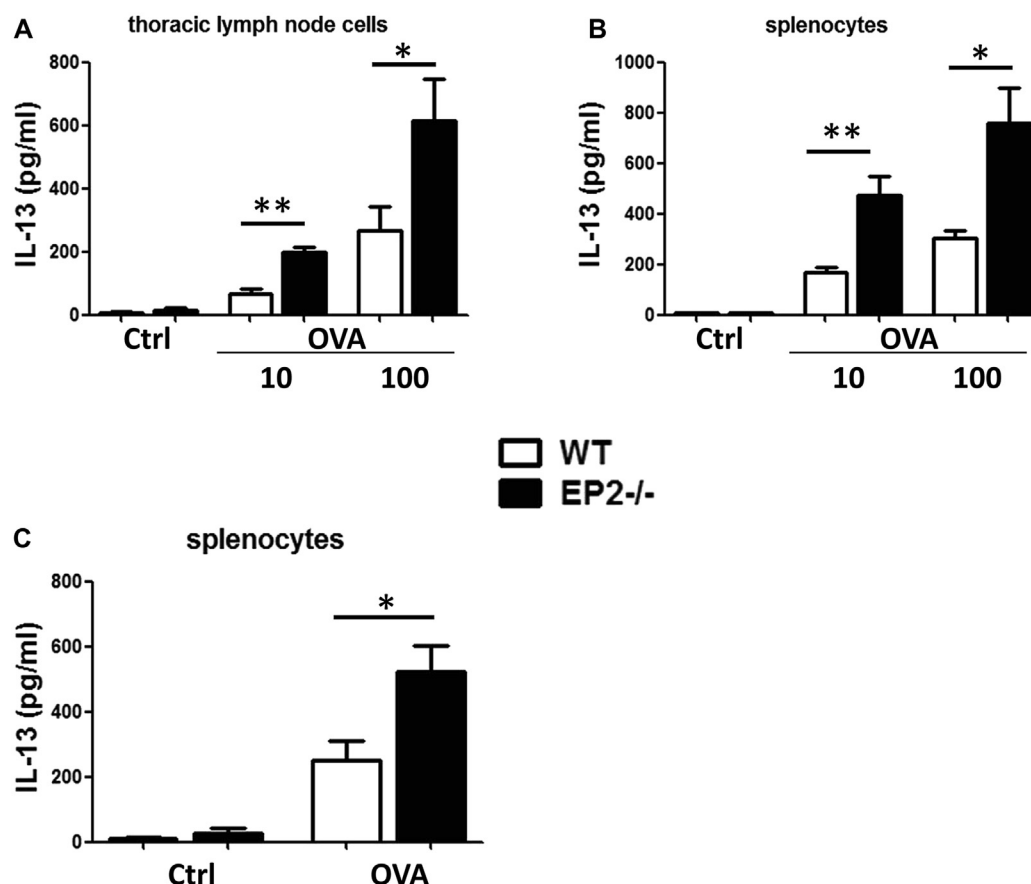


FIG 2. Augmented T_H2 responses in $EP2^{-/-}$ mice are present during the sensitization phase. **A** and **B**, Thoracic lymph node cells (Fig 2, **A**) and splenocytes (Fig 2, **B**) were isolated after the second airway challenge and cultured for 3 days in the presence of OVA. Controls were cultured without OVA ($n = 4$). **C**, Splenocytes isolated from mice after sensitization and without airway challenge were cultured for 3 days. Culture supernatants were analyzed for IL-13 by means of ELISA ($n = 4$). * $P < .05$ and ** $P < .01$.

responses in the absence of PGE_2 -EP2 signaling are not T_H2 specific. IL-4 was not detected in our *in vitro* system (data not shown), which is consistent with similar findings reported in another study of allergic asthma.²⁵ These heightened cellular responses (Fig 2, **A** and **B**) and allergic lung inflammatory responses (Fig 1) in $EP2^{-/-}$ mice do not discriminate between enhanced allergen sensitization versus enhanced airway challenge phases of this model. To specifically focus on the former, we isolated splenocytes from mice that underwent sensitization but not airway challenge and cultured them with OVA, as depicted in Fig 2, **A** and **B**. Splenocytes isolated at this point in the protocol from $EP2^{-/-}$ mice likewise exhibited greater IL-13 (Fig 2, **C**) and IFN- γ (data not shown) production than those from WT mice (Fig 2, **C**), suggesting the presence of an increased immune response during sensitization to antigen in $EP2^{-/-}$ mice.

Exogenous PGE_2 signaling strongly decreases IL-13 production *in vitro*

Data presented in Figs 1 and 2 reveal a suppressive effect of endogenous PGE_2 -EP2 signaling on antigen sensitization and subsequent airway responses. We next used pharmacologic tools applied *in vitro* to further characterize the mechanisms by which PGE_2 inhibits splenocytes isolated from sensitized WT and $EP2^{-/-}$ mice. Although PGE_2 itself was able to decrease

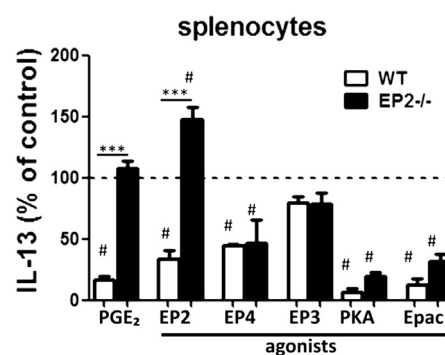


FIG 3. Exogenous PGE_2 suppresses splenocyte IL-13 production through cAMP signaling. Splenocytes from sensitized WT and $EP2^{-/-}$ mice were cultured for 3 days with 10 μ g/mL OVA in the presence of PGE_2 , EP2 agonist, EP3, EP4 agonist (all at 1 μ mol/L), or the PKA-specific cAMP analog 6-Bnz-cAMP or the Epac-specific cAMP analog 8-pCPT-2'-O-Me-cAMP (both at 500 μ mol/L). Treatments indicated with # were statistically different ($P < .001$) compared with control values. *** $P < .001$ ($n = 4$).

OVA-induced production of IL-13 by approximately 85% (Fig 3) and IFN- γ by approximately 100% (data not shown) from WT mice, it did not reduce production of both cytokines in splenocytes from $EP2^{-/-}$ mice. Exogenous administration of PGE_2 analogs selective for either EP2 or EP4 also reduced IL-13

production, whereas an EP3 agonist had no such effect. cAMP can act through either of 2 effector proteins: PKA or Epac.²⁶ cAMP analogs selective for activation of either PKA or Epac both strongly reduced IL-13 levels, indicating that both effectors can participate in the process of inhibition of antigen sensitization by PGE₂.

***In vivo* administration of a PGE₂ analog during sensitization inhibits allergic inflammation**

Misoprostol, a stable but receptor-nonspecific PGE₂ analog, was administered subcutaneously by using a regimen reported previously to assess the effects of PGE₂ on *in vivo* allergic sensitization.¹⁴ Specifically, misoprostol was administered 2 hours before and 10 hours after intraperitoneal OVA/alum injection, and 7 days later, mice underwent 2 consecutive daily airway challenges with OVA; BALF and serum samples were collected 24 hours after the second airway challenge (Fig 4, A). The potential receptor dependence of misoprostol was evaluated by determining its actions in EP2^{-/-} compared with WT mice. Misoprostol-treated WT mice showed reduced eosinophil numbers (Fig 4, B) and IL-5 and IL-13 levels (Fig 4, D) in BALF and reduced IgE levels in serum (Fig 4, E). By contrast, misoprostol's effects in EP2^{-/-} mice were less pronounced and not statistically significant (Fig 4, B-E). Mononuclear cell numbers were unaffected by misoprostol (Fig 4, C). Lymphocytes and granulocytes each accounted for less than 5% of total BALF cells, and their numbers did not differ between genotypes (data not shown). IL-13 production by cultured splenocytes isolated from mice treated with misoprostol was reduced in WT but not EP2^{-/-} mice (Fig 4, F). These experiments suggest that *in vivo* PGE₂, mainly acting through EP2, inhibits the immunologic events occurring during sensitization, and this action is sufficient to attenuate allergic inflammation after subsequent airway challenge.

PGE₂ prevents IL-13 production primarily by targeting EP2 on T cells and inhibits signal transducer and activator of transcription 6 phosphorylation

CD4⁺ T cells and CD11c⁺ DCs from OVA-sensitized spleens of WT and EP2^{-/-} mice were sorted by means of flow cytometry to high purity and cocultured in a "mix and match" design to dissect the relative roles of antigen-presenting cells versus T cells in the inhibitory actions of PGE₂ (Fig 5, A). A marked increase in IL-13 production was only observed in cocultures containing EP2^{-/-} T cells, whereas cytokine generation was not influenced by the absence of EP2 on DCs. These data strongly suggest that PGE₂ inhibition of sensitization is mediated by suppressive actions targeting EP2-bearing CD4⁺ T cells rather than DCs. Splenic CD4⁺ T cells were purified by using magnetic beads from sensitized WT and EP2^{-/-} mice and cultured for 3 days on plates precoated with CD3/CD28 antibodies to further explore the inhibitory effect of PGE₂ directed at T cells. CD4⁺ T cells from EP2^{-/-} mice exhibited significantly higher IL-13 production than did cells from WT mice (Fig 5, B). PGE₂ and the EP2 agonist inhibited IL-13 production by approximately 75% in CD4⁺ T cells from WT but not EP2^{-/-} animals; the EP3 agonist had no effect, and the effect of the EP4 agonist here was significant but less than that of the EP2 agonist (Fig 5, C). Additionally, PKA and Epac agonists both

significantly inhibited IL-13 production in T cells from both genotypes (Fig 5, C). Exogenous PGE₂ also inhibited CD4⁺ T-cell production of other T_H2 cytokines (IL-4 and IL-5), as well as of IFN- γ , but did not affect IL-17 production (data not shown). Generation of T_H2 cytokines depends on phosphorylation at residue Y641 and subsequent activation of the key transcription factor signal transducer and activator of transcription (STAT) 6.^{27,28} Interestingly, PGE₂ treatment reduced both constitutive (Fig 6, A) and IL-4-stimulated (Fig 6, B) STAT6 phosphorylation in splenic T cells isolated from sensitized mice. Together, these data suggest that CD4⁺ T cells are important targets for the inhibitory actions of PGE₂ on allergen sensitization and subsequent lung inflammation.

Adoptive transfer of CD4⁺ T cells from EP2^{-/-} mice induces a greater cytokine response in the lung

To validate the role and importance of EP2 expression on CD4⁺ T cells as a brake on allergic inflammation, we used a CD4⁺ T-cell transfer model in unsensitized mice subsequently subjected to airway challenge by using a protocol outlined in Fig 7, A. Compared with transfer of WT CD4 T cells, transfer of EP2^{-/-} CD4⁺ T cells from mice that underwent sensitization and airway challenge resulted in greater cytokine levels in the BALF of unsensitized recipient WT mice. Although the increases reached statistical significance only for the T_H2 cytokines IL-4 and IL-5, a trend toward increased levels in mice receiving EP2^{-/-} CD4⁺ T cells was also observed for IL-13, IFN- γ , and IL-17 (Fig 7, B). These data validate the important role of EP2-bearing T cells as a suppressive influence on allergic inflammation but also suggest that the inhibitory effects of PGE₂-EP2 signaling in CD4⁺ T cells might extend to polarization of T cells in general.

DISCUSSION

Exogenous PGE₂ has long been known to be a direct bronchodilator²⁹⁻³² and to protect against airway inflammatory responses to challenges, such as aspirin^{33,34} and allergen.³⁵⁻³⁷ However, this abundant prostanoid is also well known to exert pleiotropic effects on immune responses, and its influence on the initiation of the allergic response and the subsequent consequences thereof in a model of allergic asthma remains uncertain. Precedent exists for EP2 mediating suppressive actions of PGE₂ on both maturation of DCs¹⁵ and T-cell proliferation.¹⁶ Here, for the first time, we have definitively shown that PGE₂-EP2 signaling acts as an endogenous brake on the development of adaptive T_H2-polarized immunity and that this is sufficient to blunt local inflammatory responses on subsequent airway challenge. Although modest effects on DCs were observed, T cells appear to be the main target of PGE₂ action. Moreover, we have identified that PGE₂ inhibits phosphorylation of STAT6, which is a transcription factor involved in T_H2 differentiation. Such a decrease in STAT6 activation represents a possible mechanism by which PGE₂-EP2 attenuates the generation of T_H2 cytokines. T_H2 cells are widely regarded as the main producers of T_H2 cytokines and are sufficient for the induction of inflammation and the physiologic and pathologic changes associated with asthma.³⁸ Although we have not established in our study that CD4 T cells are entirely responsible for the increase in T_H2 cytokines *in vivo*, it is highly likely that they are major contributors to this phenomenon. Because effects of PGE₂ extend also to

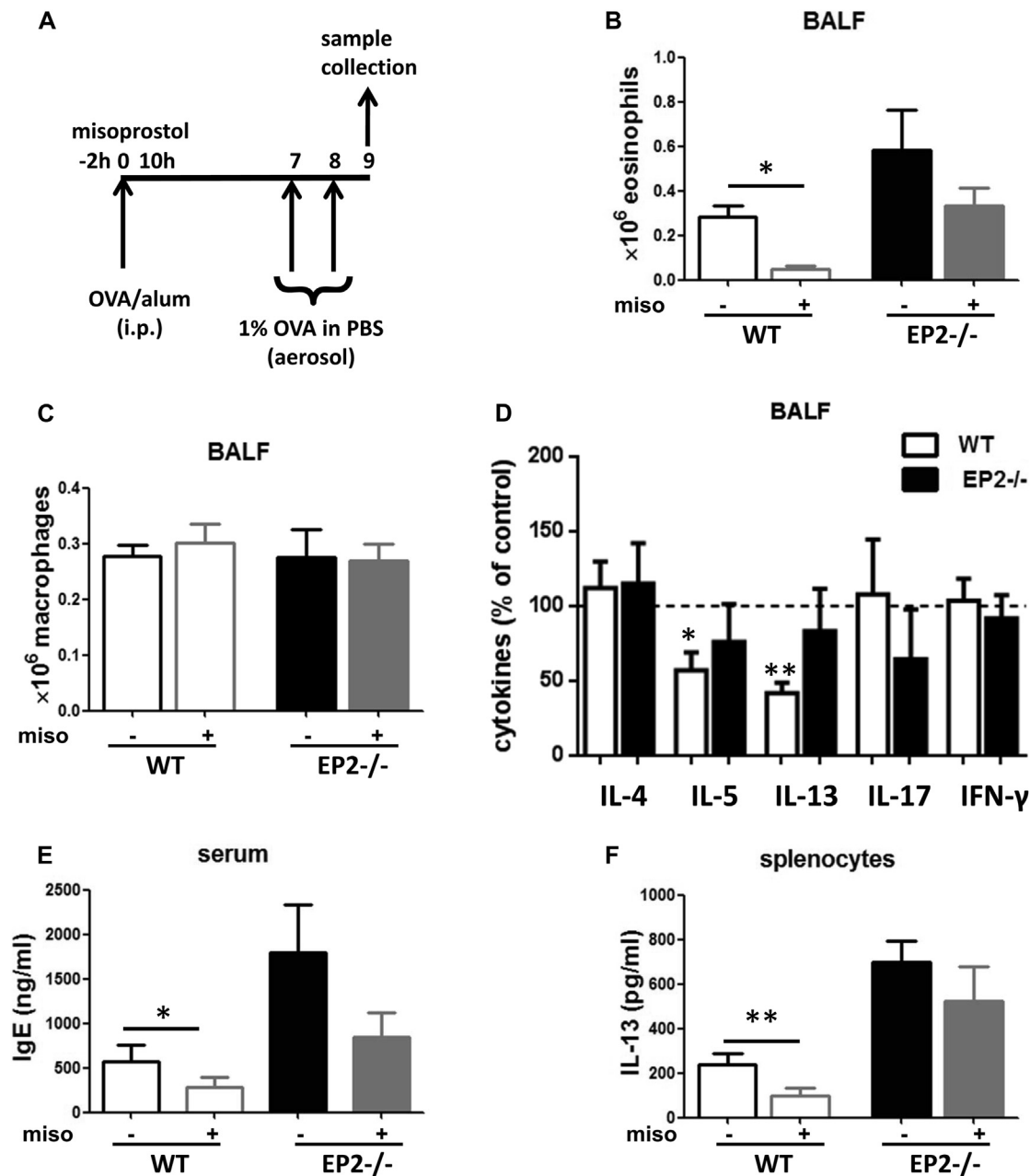


FIG 4. Subcutaneous administration of a PGE₂ analog during the sensitization phase inhibits allergic inflammation after airway challenge. **A**, Schematic representation of the misoprostol treatment protocol (50 μ g of misoprostol or DMSO vehicle administered 2 hours before and 10 hours after sensitization). **B** and **C**, Misoprostol reduces eosinophilia in WT but not EP2^{-/-} mice. **D**, Misoprostol reduces BALF IL-5 and IL-13 levels in WT but not EP2^{-/-} mice. **E**, Misoprostol reduces serum IgE levels in WT but not EP2^{-/-} mice. **F**, Splenocytes isolated from WT but not EP2^{-/-} mice treated with misoprostol have decreased IL-13 production *in vitro* (n = 4). *P < .05 and **P < .01.

other polarization states, our findings might extend beyond asthma to other conditions in which generation of polarized T cells is important.

An inhibitory effect of PGE₂ on T-cell proliferation has been very well documented.³⁹ However, its effect on T-cell cytokine secretion is much more controversial. PGE₂ has been reported to inhibit synthesis of T_H1 cytokines, such as IFN- γ .⁴⁰ By contrast, a number of studies over the years have reported that PGE₂ promotes T_H2 responses,^{18,41,42} and this conclusion has

largely been taken as axiomatic. Inhibition of T_H2 cytokines by an endogenous prostanoid was strongly suggested by studies showing their upregulation by pharmacologic inhibition or genetic deletion of COX isoenzymes in mouse models of allergic asthma.^{3-5,43-45} These experimental approaches were not designed to implicate a particular prostanoid in such suppressive actions; however, in view of its known immunosuppressive actions exerted on T cells and other cell types pertinent to asthma,^{15,16} PGE₂ is an attractive candidate to mediate this

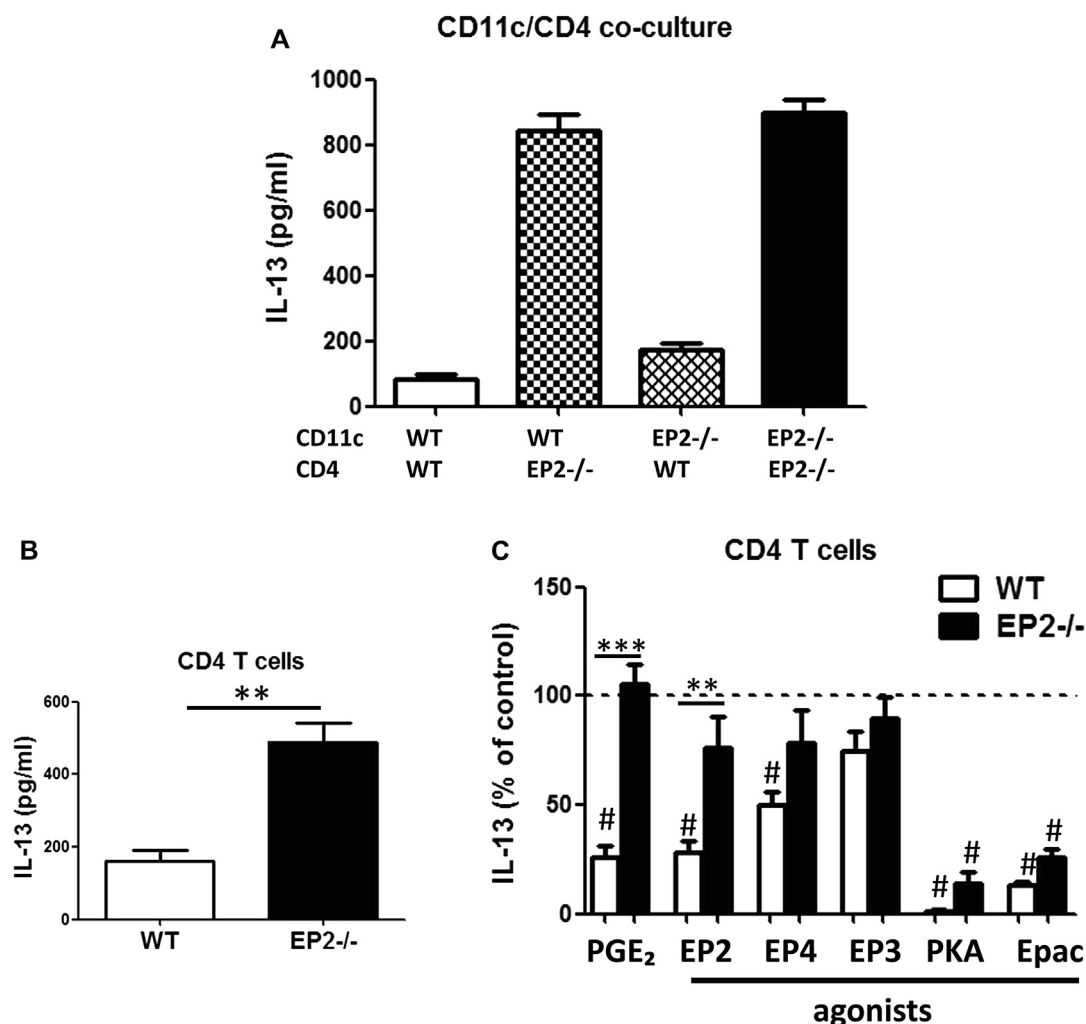


FIG 5. PGE₂ inhibits IL-13 production primarily by inhibiting T cells through EP2. **A**, EP2 deficiency on T cells primarily contributes to enhanced T_H2 responses in EP2^{-/-} mice. Purified DCs and T cells were cocultured at a ratio of 1:10 for 3 days in the presence of 100 µg/mL OVA. Data are from 1 experiment that is representative of 3 independent experiments. **B**, CD4⁺ T cells from EP2^{-/-} mice exhibited significantly higher IL-13 production than cells from WT mice. **C**, PGE₂ suppresses T_H2 polarization. Purified CD4⁺ T cells were cultured in the presence of 1 µmol/L PGE₂, EP2, EP3, or EP4 agonist or 500 µmol/L PKA-specific cAMP analog 6-Bnz-cAMP or the Epac-specific cAMP analog 8-pCPT-2'-O-Me-cAMP. #Treatments statistically different compared with control values (n = 3). **P < .01 and ***P < .001.

COX-dependent suppression of allergen sensitization. One approach to evaluating this possibility involved the use of mice deficient in the key PGE synthase microsomal PGE synthase 1. However, conflicting results were observed because allergic inflammation in *mpges*^{-/-} mice was reported to be reduced in an OVA model⁴² but increased in a house dust mite model.⁹ These conflicting findings might underscore the divergent signaling from, and biologic actions attributable to, specific EP receptors. One report used a genetic approach to address the role of specific EP receptors in allergic asthma. In that study mice deficient in each EP subtype were examined in response to OVA-induced asthma.⁴⁶ Only EP3 receptor-deficient mice exhibited increased allergic inflammation, and *in vivo* administration of an EP3 agonist was found to be protective.⁴⁶ These findings were unexpected because EP3 signaling generally reduces cAMP and typically results in stimulatory effects that oppose those elicited by EP2. We were unable to identify any such suppressive actions

of this same EP3 agonist on splenocytes or purified T cells. Here we focused on PGE₂-EP2 signaling in an OVA-induced asthma model and identified enhanced allergic inflammation in EP2^{-/-} mice. The protective role of PGE₂ in our model was achieved through a reduction in T_H2 cytokine levels. This finding is consistent with the effects of PGE₂ on IL-13 production in dispersed nasal polyp cells from patients with chronic rhinosinusitis,⁴⁷ its inhibition of T_H2 (as well as T_H1) responses in human T cells,⁴⁸ and its suppression of IL-4 production by T cells, leading to reduced IgE biosynthesis.⁴⁹ Moreover, our study is supported by results showing that both EP2 and EP4 agonists suppress OVA-induced methacholine reactivity,⁵⁰ a parameter that typically correlates with allergic inflammation.

Although our findings point to the importance of EP2 on CD4 T cells, we acknowledge that the absence of EP2 receptors on other cell types could contribute to the exaggerated allergic inflammatory phenotype observed in these mice. Addressing this

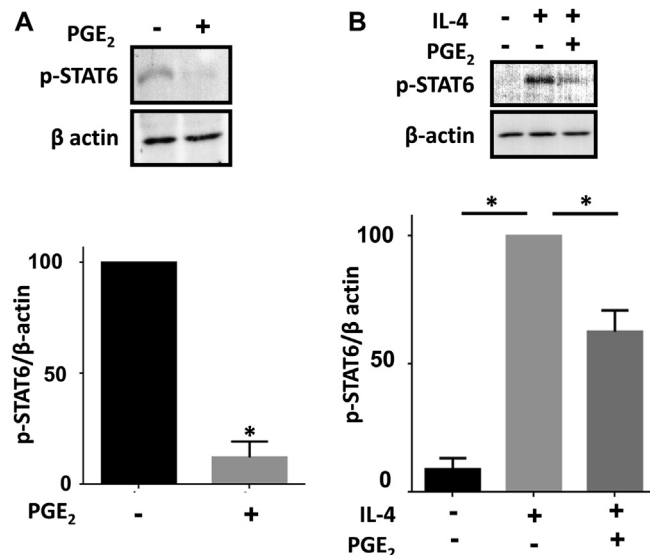


FIG 6. PGE₂ inhibits STAT6 phosphorylation (p-STAT6) in T cells. **A**, CD4 T cells cultured for 3 days in the presence of 1 μmol/L PGE₂ exhibit decreased STAT6 phosphorylation on residue Y641. **B**, CD4 T cells pretreated for 30 minutes with 1 μmol/L PGE₂ and subsequently treated for 5 minutes with recombinant IL-4 (25 ng/mL) have decreased STAT6 phosphorylation (n = 4). *P < .05.

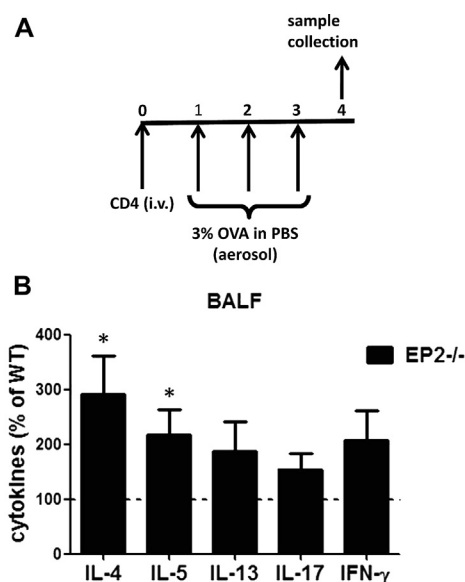


FIG 7. Adoptive transfer of CD4⁺ T cells from EP2^{-/-} mice induces increased cytokine production in WT mice. **A**, Schematic representation of the experimental protocol. *i.v.*, Intravenous. **B**, Naive WT mice were injected intravenously with 5×10^6 purified CD4⁺ T cells from asthmatic WT or EP2^{-/-} mice. Twenty-four hours later, mice were challenged with OVA, and BALF was collected 24 hours thereafter for cytokine determination by means of ELISA. WT values were set at 100% (n = 4). *P < .05.

possibility further would require mice deficient in EP2 receptors selectively on T cells. Indeed, it is well documented that PGE₂ can influence asthma pathogenesis by modulating the functions of cell types other than T cells. One example of this is its ability to directly inhibit eosinophil trafficking in the lung.^{6,51,52} Accordingly, we tested the ability of misoprostol to reduce eosinophilic lung inflammation in OVA-sensitized mice

when administered exclusively around the time of airway challenge (see Fig E1 in this article's Online Repository at www.jacionline.org). Indeed, misoprostol used in such a protocol significantly reduced T_H2 cytokine levels, as well as eosinophil numbers, in BALF but not serum IgE levels (see Fig E1). This ability of PGE₂ to attenuate lung inflammation by targeting the events driving both sensitization and challenge responses is particularly attractive from a therapeutic perspective.

PGE₂ levels are well recognized to increase in a variety of disease states, such as inflammation and cancer, and to decrease on treatment with drugs, including NSAIDs and glucocorticoids. Furthermore, EP2 expression is also subject to dynamic regulation.⁵³ Our data suggest that states characterized by deficient PGE₂-EP2 signaling might facilitate antigen sensitization. This idea is supported by epidemiologic studies indicating that the use of COX inhibitors, which include acetaminophen,⁵⁴ is associated with increased risk of allergic diseases, including asthma,⁵⁵⁻⁵⁷ and that EP2 polymorphism is associated with aspirin-induced asthma,¹⁷ a condition also typically characterized by increased sensitization to allergens. Overall, we have demonstrated that endogenous and exogenous PGE₂ suppresses allergen sensitization predominantly by ligating EP2 on T cells, thereby diminishing T_H2 polarization. Effects of exogenous PGE₂ administered exclusively during the sensitization phase were sufficient to decrease airway inflammation during the airway challenge phase 1 week later and persisted during *in vitro* culture. These data provide new insights into the regulation of immune responses and identify EP2-mediated suppression as an attractive new therapeutic strategy.

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Key messages

- PGE₂-EP2 signaling targets CD4 T cells to suppress the generation of adaptive immune responses.
- This is sufficient to inhibit allergic lung inflammation on antigen challenge.

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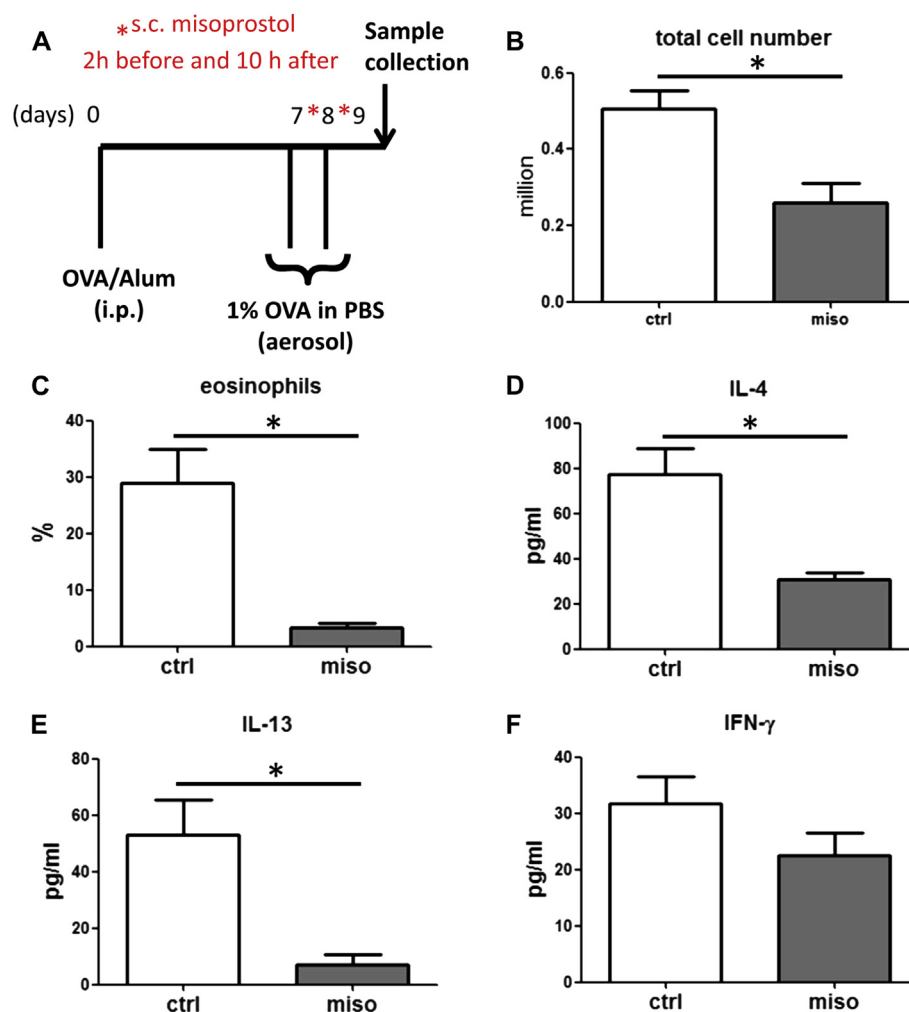


FIG E1. Subcutaneous administration of a PGE₂ analog during the airway challenge phase inhibits allergic inflammation. **A**, Schematic representation of the misoprostol treatment protocol (50 μ g of misoprostol or DMSO vehicle administered 2 hours before and 10 hours after airway challenge). *i.p.*, Intraperitoneal; *s.c.*, subcutaneous. **B-F**, Misoprostol reduces lung eosinophilia and T_H2 cytokine levels in WT mice ($n = 3$). * $P < .05$.