Prostaglandin E_2 suppresses allergic sensitization and lung inflammation by targeting the E prostanoid 2 receptor on T cells

Zbigniew Zasłona, 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) E_2 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 PGE_2 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. PGE_2 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 PGE_2-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 2014;133:379-87.)

Key words: *Asthma, allergic sensitization, prostaglandin E_2, 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) E_2, prostacyclin, PGD_2, $PGF_{2\alpha}$, and thromboxane A_2, and all of these have been implicated in various aspects of asthma pathogenesis.[6-10]

PGE_2 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 adenyl cyclase to generate the second messenger cyclic AMP (cAMP), which is typically suppressive of inflammatory cell functions. By contrast, EP3, by inhibiting adenyl cyclase, and EP1, by increasing intracellular Ca^{2+}, typically activate leukocyte functions.[11] EP2 ligation by PGE_2 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 PGE_2 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. PGE_2 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 [a]Pulmonary and Critical Care Medicine and [c]Infectious Disease, and [b]the Department of Pathology, University of Michigan Medical School.

Supported by the Deutsche Forschungsgemeinschaft (German Research Foundation, to Z.Z.), American Lung Association Senior Research Fellowships (to Z.Z., K.O., and E.B.), T32 AI007413 and American Heart Association (to R.D.-G.), and National Institutes of Health grants R01 HL94311 and R01 HL58897 (to M.P.-G.), AI065543 (to B.B.M.), AI032302 (to N.W.L.), and HD057176 (to D.M.A.).

Disclosure of potential conflict of interest: Z. Zasłona has received grants from the German Research Foundation and the American Lung Association. R. Domingo-Gonzalez has received grants from the National Institutes of Health (NIH) and the American Heart Association. B. B. Moore has received a grant from the NIH. M. Peters-Golden has received grants from the NIH, the American Lung Association, Nycomed/Takeda, and Actelion; was formerly on the respiratory advisory board for Merck and Company; has consultant arrangements with Nycomed/Takeda, GlaxoSmithKline, and Sanofi-Aventis; has received payment for lectures from Merck and Company; and shares a patent with the University of Michigan on intrapulmonary administration of LTB_4 to boost immunity, licensed to Sweden AB, and receives royalties from this patent. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication March 21, 2013; revised July 24, 2013; accepted for publication July 31, 2013.

Available online September 24, 2013.

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

http://dx.doi.org/10.1016/j.jaci.2013.07.037

Abbreviations used
BALF: Bronchoalveolar lavage fluid
cAMP: Cyclic AMP
DC: Dendritic cell
DMSO: Dimethyl sulfoxide
EP: E prostanoid
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

proliferation[19] and MHC class II expression[20,21] also endows PGE_2 with the potential to interfere with adaptive immunity. In this study we sought to specifically interrogate the role of the PGE_2-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 PGE_2-EP2 signaling in allergic asthma and further identify EP2 on $CD4^+$ T cells as the critical target mediating PGE_2-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), PGE_2, and PGE_2 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 PGE_2 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′-cyclicmonophosphate) 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 2×10^5 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 PGE_2 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 Biotech). 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

PGE_2-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 PGE_2-EP2 signaling in a model of OVA-induced allergic asthma.[24] After sensitization and