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The PGI₂ Analog Cicaprost Inhibits IL-33–Induced Th2 Responses, IL-2 Production, and CD25 Expression in Mouse CD4⁺ T Cells

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IL-33 has pleiotropic functions in immune responses and promotes the development of allergic diseases and asthma. IL-33 induces Th2 differentiation and enhances type 2 cytokine production by CD4⁺ T cells. However, the regulation of IL-33–driven type 2 cytokine responses is not fully defined. In this study, we investigated the effect of PGI₂, a lipid mediator formed in the cyclooxygenase pathway of arachidonic acid metabolism, on naive CD4⁺ T cell activation, proliferation, and differentiation by IL-33. Using wild-type and PGI₂ receptor (IP) knockout mice, we found that the PGI₂ analog cicaprost dose-dependently inhibited IL-33–driven IL-4, IL-5, and IL-13 production by CD4⁺ T cells in an IP-specific manner. In addition, cicaprost inhibited IL-33–driven IL-2 production and CD25 expression by CD4⁺ T cells. Furthermore, IP knockout mice had increased IL-5 and IL-13 responses of CD4⁺ T cells to Alternaria sensitization and challenge in mouse lungs. Because IL-33 is critical for Alternaria–induced type 2 responses, these data suggest that PGI₂ not only inhibits IL-33–stimulated CD4⁺ Th2 cell responses in vitro but also suppresses IL-33–induced Th2 responses caused by protease-containing allergens in vivo. The Journal of Immunology, 2018, 201: 1936–1945.

Lipid molecules such as PGs formed in the cyclooxygenase pathway of arachidonic acid metabolism have regulatory functions in immune responses and inflammation (6–9). Prostaglandin I₂ (PGI₂), also known as prostacyclin, suppressed Th2 cytokine (IL-5 and IL-13) expression, eosinophilia, and mucus production in the lung through the PGI₂ receptor (IP) signaling pathway in a mouse model of OVA-induced allergic inflammation (10–13). Recently, we reported that IP deficiency abrogated OVA-induced immune tolerance in mice, further supporting the modulatory functions of PGI₂ not only on immune responses but also on immune tolerance (14). In vitro, the PGI₂ analogs cicaprost and iloprost inhibited the production of the effector cytokines IL-4 and IL-5 by Th2 cells that had been differentiated under IL-4 and anti–IFN-γ conditions (15). However, the effect of PGI₂ on IL-33–driven Th2 polarization has not been reported.

In this study, we tested the hypothesis that PGI₂ inhibits IL-33–induced CD4⁺ T cell activation, proliferation, and type 2 cytokine expression. We found that the PGI₂ analog cicaprost suppressed IL-33–induced production of IL-4, IL-5, and IL-13 by wild-type (WT) CD4⁺ T cells but not by IP knockout (KO) CD4⁺ T cells. In addition, cicaprost signaling through IP decreased IL-33–induced IL-2 production and CD25 surface expression by CD4⁺ T cells. The suppressive effect of the PGI₂ analog cicaprost on IL-33–driven Th2 responses suggests that PGI₂ limits the development of type 2 immune responses caused by IL-33–stimulating Ags such as protease-containing allergens. This notion is supported by our findings that IP KO mice had increased CD4⁺ cell IL-5 and IL-13 responses in the model of Alternaria–induced lung inflammation.

Materials and Methods

**Mice**

WT BALB/c mice were obtained from The Jackson Laboratory (Bar Harbor, ME). IP KO mice on a C57BL/6 background were generated by homologous recombination in embryonic stem cells and kindly provided by Dr. G. FitzGerald (16) at the University of Pennsylvania. The IP KO...
mice were backcrossed to a BALB/c background for 10 generations. Age-matched WT and IP KO mice were used at 8–12 wk old. Animal experiments were reviewed, approved by the Institutional Animal Care and Use Committee at Vanderbilt University, and were conducted according to the Guidelines for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources of the National Research Council.

**CD4+ T cell culture**

CD4+ T cells were purified from the splenic cells of WT or IP KO mice by a mouse naive CD4+ T cell isolation kit (catalog no. 130-106-643; Miltenyi Biotec, Auburn, CA). An anti-CD11c Ab was included in this kit to deplete dendritic cells. The isolated CD4+CD62L+ T cell population contains 97.5% CD3+CD4+ cells as determined by flow cytometry. The purified CD4+ T cells were resuspended at 1 × 10^6 cells/ml in RPMI 1640 medium (Mediatech, Herndon, VA) supplemented with 10% FBS (HyClone, Logan, UT), 4 mM of t-glutamine, 1 mM of sodium pyruvate, 55 mM of 2-ME, 10 mM of HEPES, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were stimulated with plate-bound anti-CD3 (10 μg/ml) and anti-CD28 (2 μg/ml; BD Biosciences, San Diego, CA) in 96-well flat-bottom plates and treated with IL-33 (20 ng/ml; PeproTech, Rocky Hill, NJ) plus cicaprost (a generous gift provided by Dr. M. Huebner, Bayer HealthCare, Berlin, Germany) at various concentrations or vehicle (water) control. The cells were cultured for 3 d.

Cell culture supernatant was collected at day 2 for IL-2 measurement because IL-2 production by activated CD4+ T cells peaks at day 2 (W. Zhou, unpublished observations). At day 3, the culture supernatant was harvested for IL-4, IL-5, and IL-13 measurement.

**RT-PCR**

Naive CD4+ T cells and CD4+ T cells activated under the conditions with anti-CD3 and anti-CD28 in the absence or presence of IL-33 for 3 d were used for RT-PCR to determine IP, COX-2, PGI2 synthase (PGIS), and β-actin expression. TaqMan Real-Time PCR Assays (Thermo Fisher Scientific, assay identifiers: IP, Mm00801939_m1; COX-2, Mm00478374_m1; PGIS, Mm00447271_m1; and β-actin, Mm02619580_g1) were used for the experiments. LPS-stimulated mouse lung tissue was used as positive control of these target genes.

**ELISA**

IL-2, IL-4, IL-5, and IL-13 in the culture supernatant were measured by DuoSet ELISA kits from R&D Systems according to the manufacturer’s instructions. Measurements below the limit of detection were assigned a value of half the lower limit of detection for the purpose of statistical analyses.

**Flow cytometry**

At day 3 of cell culture, for intracellular IL-4, IL-5, and IL-13 flow cytometry, cells were stimulated with PMA (10 ng/ml), ionomycin (1 μM), and GolgiStop (2.25 mM monensin; BD Biosciences) for 4–6 h. The cells were stained with LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific), fixed, and permeabilized. The cells were then stained with fluorochrome-labeled Abs against CD4 (BioLegend), IL-4 (BD Biosciences, San Jose, CA), IL-5 (BD Biosciences), IL-13 (BioLegend), Gata3 (Affymetrix, Santa Clara, CA), CD25 (BD Biosciences), and TSLP (BioLegend). For cell counting purposes, 123count eBeads (Affymetrix) were added to the cell solution. An LSR II flow cytometer (BD Biosciences) was used for flow cytometry, and the data were analyzed with FlowJo software (FlowJo, Ashland, OR). For cell proliferation assay, naive CD4+ T cells were labeled with CFSE (0.5 μM) before cell activation and treatment. At day 3, DAPI and 123count eBeads (Affymetrix) were added to the cell solution for dead cell staining and cell counting purposes by flow cytometry. CFSE intensity of the cultured cells was determined by flow cytometry. Cell division index was calculated with FlowJo software.

**NFAT function assay**

Cignal Lenti NFAT luciferase reporter system (Qiagen, Hilden, Germany) was used for determining the effect of cicaprost on NFAT function, according to manufacturer’s instructions. Briefly, purified naive CD4+ T cells were transduced with Cignal Lenti NFAT or Cignal Lenti GFP

![FIGURE 1](http://www.jimmunol.org/) Cnicaprost decreased IL-33–induced IL-4, IL-5, and IL-13 expression by CD4+ T cells. Naive CD4+ T cells from WT and IP KO mice were stimulated with anti-CD3 and anti-CD28 Abs in the absence or presence of IL-33 and were treated with vehicle or cicaprost for 3 d. The levels of (A) IL-4, (B) IL-5, and (C) IL-13 in the culture supernatant were determined by ELISA. Data are a combination of three experiments and presented as mean ± SEM. n = 9, *p < 0.05.
reporter vectors in the presence of SureENTRY Transduction Reagent and cultured for 24 h to ensure lentivirus integration. The cells were washed and treated with vehicle or cicaprost in the absence or presence of IL-33 for 2 d. Lenti NFAT–transduced cells were harvested, and the cell pellets were used for luciferase assay with a Luciferase Assay System (Promega, Madison, WI) to determine the NFAT activity. Lenti GFP–transduced cells were stained with dead cell dye and analyzed by flow cytometry to determine the transduction efficiency of live cells. We found that ∼2% of transduced cells were GFP+.

Alternaria extract challenge in vivo
WT and IP KO mice were anesthetized with ketamine/xylazine and then challenged intranasally with either Alternaria extract (5 μg) or heat-inactivated (120°C for 2 h) Alternaria extract (5 μg) (Greer Laboratories, Lenoir, NC) in 100 μl PBS four times on days 0, 3, 6, and 9. Twenty-four hours after the last challenge, the mouse lungs were harvested and digested. Lung cells were stimulated with PMA (10 ng/ml), ionomycin (1 μM), and GolgiStop (2.25 μM monensin; BD Biosciences) for 4–6 h. Cell surface marker and intracellular cytokine staining was performed to enumerate IL-5+CD4+ T cells and IL-13+CD4+ T cells. Lung homogenate was used for determining the levels of IL-5 and IL-13 by ELISA.

**Statistical analysis**
The results were presented as mean ± SEM. Statistical analyses were conducted by using Student t test or one-way ANOVA with a Bonferroni post hoc test for Fig. 8.
Results
The PG\textsubscript{I\textalpha} analog cicaprost decreased IL-33–induced type 2 cytokine production by CD4\textsuperscript{+} T cells

To determine the effect of PG\textsubscript{I\textalpha} signaling on IL-33–induced Th2 differentiation, naive CD4\textsuperscript{+} T cells of WT and IP KO mice, both on a BALB/c background, were activated with anti-CD3 and anti-CD28 either with or without IL-33 and treated with cicaprost or vehicle for 3 d. We found that naive and activated CD4\textsuperscript{+} T cells expressed IP receptor as determined by RT-PCR (Supplemental Fig. 1), providing a molecular basis of T cell responsiveness to cicaprost treatment. Activated CD4\textsuperscript{+} cells, but not naive CD4\textsuperscript{+} cells, expressed COX-2, whereas neither naive nor activated CD4\textsuperscript{+} cells expressed PGIS (Supplemental Fig. 1). As shown in Fig. 1, treatment of the cells with IL-33 significantly increased the production of the type 2 cytokines IL-4, IL-5, and IL-13 compared with the cell culture without IL-33. In the presence of IL-33, cicaprost dose-dependently decreased IL-4, IL-5, and IL-13 production by WT CD4\textsuperscript{+} T cells, suggesting that PGI\textsubscript{2} has an inhibitory effect on Th2 differentiation and type 2 cytokine production. Cicaprost did not change the production of IL-4, IL-5, or IL-13 by IP KO CD4\textsuperscript{+} T cells, indicating that cicaprost-mediated inhibition of type 2 cytokine production is dependent on IP signaling.

To determine the effect of cicaprost on type 2 cytokine expression at a single-cell level, we activated and treated naive CD4\textsuperscript{+} T cells with vehicle or cicaprost for 3 d and stimulated the cells with PMA and ionomycin in the presence of GolgiStop for intracellular cytokine measurement by flow cytometry. We found that IL-33 increased the numbers of IL-4\textsuperscript{+}CD4\textsuperscript{+}, IL-5\textsuperscript{+}CD4\textsuperscript{+}, and IL-13\textsuperscript{+}CD4\textsuperscript{+} cells compared with the cell culture in the absence of IL-33 for both WT and IP KO T cells (Fig. 2). In the presence of IL-33, cicaprost dose-dependently decreased total numbers of IL-4\textsuperscript{+}CD4\textsuperscript{+}, IL-5\textsuperscript{+}CD4\textsuperscript{+}, and IL-13\textsuperscript{+}CD4\textsuperscript{+} cells compared with vehicle control in WT T cell culture but not in IP KO T cell culture (Fig. 2). These results indicate that the inhibitory effect of cicaprost on CD4\textsuperscript{+} T cell type 2 cytokine expression is dependent on the IP signaling pathway.

Cicaprost did not affect the percentages of IL-4\textsuperscript{+}CD4\textsuperscript{+}, IL-5\textsuperscript{+}CD4\textsuperscript{+}, and IL-13\textsuperscript{+}CD4\textsuperscript{+} cells. Neither did cicaprost affect mean fluorescence intensity (MFI) of IL-4, IL-5, and IL-13 of the cells in these experiments (Supplemental Fig. 2). These data suggest that cicaprost inhibited IL-33–induced Th2 responses by limiting the total number of Th2 cells rather than by affecting type 2 cytokine expression at a single-cell level.

*Cicaprost decreased Gata3 expression in CD4\textsuperscript{+} T cells*

The differentiation of naive CD4\textsuperscript{+} T cells into Th2 cells under IL-4 culture conditions requires the expression of the transcription factor Gata3 (17). In this study, IL-4 was endogenously produced in the presence of IL-33. To investigate whether Gata3 plays a role in the inhibitory effect of cicaprost on IL-33–induced type 2
cytokine production, we determined the protein expression of Gata3 in CD4+ T cells activated with IL-33 and treated with cicaprost at various concentrations or vehicle for 3 d. As shown in Fig. 3, we found that cicaprost at 1000 nM significantly decreased total numbers, the percentages, and Gata3 MFI of Gata3-expressing CD4+ T cells from WT mice but not IP KO mice. Therefore, the IP-dependent suppression of type 2 cytokine production by cicaprost was associated with decreased Gata3 protein expression, suggesting that PGI2/IP signaling inhibits Gata3 expression and suppresses Th2 differentiation.

Cicaprost decreased IL-2 production, NFAT activation, and CD4+ T cell activation and proliferation

In vitro activation of mouse naive CD4+ T cells results in the expression of IL-2, a growth factor critical for cell survival and proliferation (18). To investigate whether cicaprost inhibits IL-33-induced type 2 cytokine production by suppressing CD4+ T cell IL-2 production and cell activation, we measured IL-2 levels in the culture supernatant. We found that IL-33 significantly increased CD4+ T cell IL-2 expression (Fig. 4A). Cicaprost dose-dependently suppressed IL-2 production by WT CD4+ T cells but not by IP KO CD4+ T cells, indicating that the suppressive effect of cicaprost is dependent on IP signaling (Fig. 4A).

To determine whether the suppressive effect of cicaprost on IL-2 production is associated with the inhibition of NFAT, a transcription factor that binds to the IL-2 gene promoter and activates IL-2 gene expression, we performed an NFAT functional assay with an NFAT luciferase reporter lentiviral vector. We found that cicaprost significantly suppressed NFAT function in WT cells but not IP KO cells after 3 d of cell culture (Fig. 4B). These results suggest that cicaprost inhibits IL-2 production by attenuating NFAT activation.

To further analyze the effect of cicaprost on cell proliferation, naive CD4+ T cells were CFSE labeled, activated with anti-CD3 and anti-CD28, and treated with IL-33 plus either vehicle or cicaprost. Cell proliferation was determined by CFSE dilution. As shown in Fig. 5, cicaprost at 1000 nM significantly decreased the cell division index and the total number of live cells of WT CD4+ T cells but not IP KO CD4+ T cells (Fig. 5). These data indicate that cicaprost inhibited cell activation and proliferation in an IP-dependent manner.

Cicaprost decreased IL-5 and IL-13 production by CD4+ T cells in the presence of exogenous IL-2

IL-2 not only stimulates CD4+ T cell survival and expansion but also promotes Th2 differentiation in the presence of IL-4 and anti–IFN-γ (19). The correlation between IL-2 inhibition and type 2 cytokine suppression in cicaprost-treated cells suggests that decreased IL-2 expression may be a mechanism by which cicaprost subsequently inhibits IL-33–induced type 2 cytokine production. To test this hypothesis, we added IL-2 to the cell cultures, reasoning that if cicaprost inhibits type 2 cytokine production by affecting IL-2 production, addition of exogenous IL-2 should attenuate or abrogate the effect of cicaprost. We found that cicaprost still significantly inhibited IL-5 and IL-13 production in the presence of either 10 or 50 ng/ml exogenous IL-2 (Fig. 6A, 6B), suggesting that other mechanisms downstream of IL-2 expression, such as the IL-2R signaling pathway, are involved in the inhibitory effect of cicaprost on type 2 cytokine production.

Cicaprost decreased CD25 expression on CD4+ T cells

to determine whether cicaprost attenuates the IL-2 signaling pathway, CD4+ T cells activated and treated with either cicaprost or vehicle for 3 d were stained with fluorochrome-labeled anti-CD25 Ab and analyzed for the expression of IL-2Rα-chain (CD25) by flow cytometry. CD25 is a high-affinity IL-2R subunit. The inducible expression of CD25 on naive CD4+ T cells enables fully functional IL-2Rs for the responses to IL-2 and is critical for naive CD4+ T cell activation. We found that cicaprost dose-dependently decreased total numbers and percentages of CD25-expressing cells, and CD25 MFI compared with vehicle control (Fig. 7A–D). As a comparison, cicaprost did not change
percentages of TSLPR-expressing cells and TSLPR MFI in WT or IP KO T cell culture in the presence of IL-33 (data not shown).

IP deficiency increased CD4+ T cell IL-5 and IL-13 responses in vivo

To investigate the in vivo relevance of the IP-dependent inhibitory effect of cicaprost on IL-33–enhanced Th2 responses, we challenged WT and IP KO mice with *Alternaria* extract, a protease-containing substance that has been used as a model allergen to induce lung inflammation in mice (2). It has been shown that IL-33R signaling is required for *Alternaria*-induced type 2 responses and exacerbation of allergic inflammation, eosinophilia, and IL-13 production in the lung (21, 22). We used a 10-d allergen sensitization and challenge model in which the adaptive immune system and CD4+ T cells are activated. We found that IP KO mice had significantly increased numbers of IL-5+CD4+ T cells and IL-13+CD4+ T cells in the lung and greater protein levels of IL-5 and IL-13 in the lung homogenate (Fig. 8).

**FIGURE 5.** Cicaprost inhibited CD4+ T cell proliferation. Naive CD4+ T cells from WT or IP KO mice were CFSE labeled and stimulated with anti-CD3 and anti-CD28 Abs in the absence or presence of IL-33 and were treated with vehicle or cicaprost for 3 d. (A) Cell division was analyzed by flow cytometry. (B) Cell division index. (C) Total numbers of live cells. Data are a combination of five (B and C) experiments and presented as mean ± SEM. n = 15. *p < 0.05.

**FIGURE 6.** Cicaprost decreased type 2 cytokine production by CD4+ T cells in the presence of exogenous IL-2. Naive CD4+ T cells of WT mice were stimulated with anti-CD3 and anti-CD28 Abs in the absence or presence of IL-33 and IL-2 and were treated with vehicle or cicaprost for 3 d. The levels of (A) IL-5 and (B) IL-13 in the culture supernatant were determined by ELISA. Data are a combination of four experiments and presented as mean ± SEM. n = 8–11. *p < 0.05.
These results indicated that IP deficiency augmented type 2 CD4+ T cell responses and type 2 cytokine responses to *Alternaria* challenge, suggesting that endogenous PGI2 inhibits IL-33–enhanced Th2 responses.

**Discussion**

The discovery of IL-33–induced Th2 polarization reveals an alternative Th2 differentiation pathway (5). We have previously published findings on the regulation of allergic responses and inflammation by lipid mediators formed in arachidonic acid metabolism (10, 14, 23). We have shown that PGI2 and IP signaling inhibit the effector cytokine production by Th2 cells that had been differentiated under classical Th2 conditions with IL-4 and anti-IFN-γ (15). In this study, we demonstrate that the PGI2 analog cicaprost and IP signaling negatively regulated IL-33–driven Th2 responses and...
reduced IL-4, IL-5, and IL-13 production and Gata3 protein expression in CD4+ T cells in a dose-dependent manner. The inhibitory effect of cicaprost is associated with decreased NFAT activation, IL-2 expression, and CD4+ T cell proliferation and correlated with lower surface expression of IL-2R \( \alpha \)-chain (CD25) in CD4+ T cells. The data presented in this article suggest that IP signaling restrains IL-33–driven alternative Th2 responses in part by inhibiting IL-2 production and suppressing IL-2R \( \alpha \)-chain expression. In addition, we demonstrated that IP KO mice had increased adaptive immunity, Th2 cell numbers, and type 2 cytokine responses in protease-containing, allergen-induced lung inflammation. As IL-33 has been shown to be critical for Alternaria-induced lung type 2 responses (2, 21), these data suggest an in vivo inhibitory effect of PGI2 on IL-33–induced Th2 responses. To our knowledge, this is also the first report that PGI2 signaling inhibits Th2 immune responses in an allergen challenge model that does not use aluminum hydroxide as an adjuvant, increasing the potential clinical relevance of the inhibitory effect of PGI2 and its analogs in chronic allergic diseases such as asthma.

Naive CD4+ T cells activated with anti-CD3 and stimulated by IL-33 produced IL-5 and IL-13 but not IL-4 (5). In our study, we found that IL-33 stimulated not only IL-5 and IL-13 expression but also IL-4 production by either naive WT BALB/c or IP KO CD4+ T cells after activation with anti-CD3 and anti-CD28. The difference in the findings may be explained by variations in the presence of costimulatory signals in our study and strength of TCR stimulation. In addition to studies focusing on the stimulatory functions of IL-33 on innate and adaptive type 2 responses (5, 24), more recent reports showed that IL-33 can act as an enhancer for effector cytokine production by Th1, Th17, and regulatory T cells (25–27). Our data in this article demonstrate that the PGI2 analog cicaprost and IP signaling restrain IL-33–induced Th2 cell expansion and type 2 cytokine production.

Further research is needed to determine the effect of PGI2 on IL-33–mediated effects on other CD4+ T cell subsets.

IL-2 is a critical cytokine for CD4+ T cell activation and clonal expansion through autocrine and paracrine signaling. We demonstrate in this study that IL-33 significantly enhanced IL-2 production in WT and IP KO CD4+ T cells, and cicaprost dose-dependently inhibited IL-33–induced IL-2 production in WT CD4+ T cells but not in IP KO CD4+ T cells. Cicaprost also suppressed the IL-2R \( \alpha \)-chain CD25 expression. The downregulation of both IL-2 and IL-2R may contribute to cicaprost-mediated suppression of IL-2R signaling and Th2 differentiation. The suppressive effect of PGI2 on CD25 expression and IL-2R signaling has been reported in several studies. PGE2 inhibited IL-2 production and CD25 expression in human and bovine CD4+ T cells (28–31). The cAMP-elevating agents forskolin and dbcAMP had similar inhibitory effects on IL-2 and CD25 expression by human CD4+ T cells (28–31). In a human cell culture study, the PGI2 analog iloprost inhibited CD25 expression by in vitro–polarized regulatory T cells (32).

We found that cicaprost treatment decreased Gata3 expression in an IP-dependent manner, suggesting that cicaprost may
inhibit Th2 responses mediated by the Gata3 signaling pathway. Kurowska-Stolarska et al. (5) reported that IL-33 by itself did not strongly induce Gata3 expression. IL-33 enhanced IL-5 and IL-13 expression in IL-4 KO and STAT6 KO CD4+ T cells, suggesting that the augmentation of IL-5 and IL-13 production by IL-33 is independent of IL-4 and STAT6. However, in the presence of endogenous IL-4 as shown in this study, Gata3 protein was expressed at a high level and is likely to play an enhancing role in Th2 differentiation. Kurowska-Stolarska et al. (5) showed that IL-33 alone did not markedly increase GATA3 mRNA levels. Whether Gata3 is dispensable for IL-33–induced Th2 differentiation remains to be investigated. In this study, IL-33 did not further increase the levels of Gata3 expression; however, this does not exclude the possibility that cicaprost inhibits type 2 cytokine expression in the presence of endogenous IL-4 partially by suppressing Gata3 expression.

By using WT and IP KO mice, we were able to demonstrate that cicaprost-mediated inhibition of IL-33–induced CD4+ Th2 cytokine production, IL-2 production, and CD25 expression is IP dependent. IL-33 plays an important role in the development of allergic diseases induced by protease-containing allergens such as *Alternaria alternata* (2, 34). PGI2 analog–mediated inhibition of IL-33–induced Th2 responses in vitro suggests that PGI2 may limit Th2 differentiation and allergy development induced by protease-containing allergens in vivo. In a mouse model of *Alternaria*-induced lung inflammation (10-d protocol that induced adaptive immune responses), we found that IP KO mice had significantly augmented IL-5 and IL-13 responses and increased the numbers of IL-5+ CD4+ cells and IL-13+ CD4+ cells in the lung. These data suggest that PGI2 signaling through IP attenuates IL-33–induced Th2 cytokine responses in adaptive immune cells. We have recently reported that cicaprost inhibited IL-33–induced cytokine responses of type 2 innate lymphoid cells (ILC2) (33). Using a mouse model of *Alternaria* extract–induced lung inflammation (short protocol, 4 d of *Alternaria* challenge), we revealed that endogenous PGI2 has an inhibitory function on ILC2 (33). We demonstrated that IP deficiency resulted in greater ILC2 responses compared with ILC2 responses in WT mice, and conversely, cicaprost inhibited ILC2 responses in WT mice (33). Based on our previously published findings and the results in this study, it appears that PGI2 and IP receptor signaling limits IL-33– and IL-4–induced Th2 cell responses as well as ILC2 function, highlighting the broad regulatory properties of PGI2 in adaptive and innate immunity and inflammation.

Taken together, the data presented in this article indicate that the PGI2 analog cicaprost significantly downregulates IL-33–induced Th2 differentiation and type 2 cytokine production, likely via suppressing IL-2 production, CD25 expression, and Gata3 expression. PGI2 and its analog iproprit are Food and Drug Administration–approved drugs for the clinical use to treat pulmonary hypertension (35) and could be a potential therapeutic drug option for IL-33–related type 2 immune disorders such as allergic diseases and asthma.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


Fig. S1. Activated CD4 T cells expressed COX-2 and IP, but not PGI₂ synthase (PGIS). Naïve CD4 T cells were activated with anti-CD3 and anti-CD28 in the absence or presence of IL-33 for 3 days. The cells were harvested and cellular mRNA was prepared for RT-PCR analyses of target gene expression.
Fig. S2. Cicaprost did not change the percentage and mean fluorescence intensity (MFI) of IL-4, IL-5 and IL-13 in CD4+ T cells. Naïve CD4+ T cells from WT and IP KO mice were stimulated with anti-CD3 and anti-CD28 Abs in the absence or presence of IL-33 and treated with vehicle or cicaprost for 3 days. The cells were then stimulated with PMA and ionomycin in the presence of GolgiStop for 4h before stained and analyzed by flow cytometry. The cells were gated for lymphocytes, single cells, and live cells for analyses of IL-4, IL-5, or IL-13 expression. A. The percentages of IL-4+CD4+, IL-5+CD4+, and IL-13+CD4+ cells. B. MFI of IL-4, IL-5 or IL-13. Data are combined of 2 experiments and presented as mean ± SEM. *, p < 0.05, n=6.