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Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3

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Prostaglandins, including PGD_2 and PGE_2, are produced during allergic reactions. Although PGD_2 is an important mediator of allergic responses, aspirin-like drugs that inhibit prostaglandin synthesis are generally ineffective in allergic disorders, suggesting that another prostaglandin-mediated pathway prevents the development of allergic reactions. Here we show that such a pathway may be mediated by PGE_2 acting at the prostaglandin E receptor EP3. Mice lacking EP3 developed allergic inflammation that was much more pronounced than that in wild-type mice or mice deficient in other prostaglandin E receptor subtypes. Conversely, an EP3-selective agonist suppressed the inflammation. This suppression was effective when the agonist was administered 3 h after antigen challenge and was associated with inhibition of allergy-related gene expression. Thus, the PGE_2-EP3 pathway is an important negative modulator of allergic reactions.

Allergy, especially type I allergy such as bronchial asthma and allergic rhinoconjunctivitis, is a principal health problem, and the prevalence of allergy has increased during the past two decades[1]. Affected people have a high titer of immunoglobulin E (IgE) antibodies specific for allergens such as those associated with house dust mites and plant pollen[2]. Exposure of such people to these allergens induces the activation of mast cells through crosslinking of IgE receptors on the cell surface mediated by antigen-antibody binding. Activated mast cells release allergic mediators such as histamine, cysteinyl leukotrienes and various cytokines. These substances not only mediate acute allergic responses but also induce the late phase of inflammation by stimulating local synthesis of various chemokines such as CCL11 (eotaxin) and CCL17 (TARC), which recruit T helper type 2 lymphocytes and eosinophils[3–5]. Repeated exposure to allergens enhances inflammation characterized by infiltration of these cells and can cause tissue remodeling. However, how this course of responses is regulated and why it becomes exaggerated in some populations remains unknown.

Prostaglandins, including PGD_2 and PGE_2, are produced in substantial amounts during allergen exposure and disease development[4–7]. Prostaglandins act at specific cell surface receptors to elicit their own actions. Whereas PGD_2 acts at the prostaglandin D receptor, PGE_2 acts at four subtypes of prostaglandin E receptor, EP1–EP4 (ref. 8), which are encoded by *Ptger1–Ptger4*. Studies examining the function of PGD_2 in allergic asthma using mice deficient in the prostaglandin D receptor found that PGD_2 mediates this disease[9]. This view has been supported by a genetic study in humans identifying the gene encoding PGD_2 as an 'asthma susceptibility gene'[10]. If PGD_2 were the only prostaglandin important in allergy, aspirin-like nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin synthesis would be expected to suppress allergic reactions. However, these drugs are generally ineffective in the treatment of allergic disorders. In contrast, they induce life-threatening attacks in about 10% of adults with asthma[11] and aggravate nasal polyposis of allergic rhinitis[12]. Patients who develop aspirin-induced asthma also manifest other symptoms of immediate hypersensitivity such as rhinorrhea, conjunctival irritation and flushing of the head and neck. These observations suggest the existence of a prostaglandin-dependent process that suppresses type I allergic reactions in general. Here, we have examined mice deficient in each prostaglandin E receptor subtype individually and have used an EP3-selective agonist to identify this protective prostaglandin pathway. We show that the PGE_2-EP3 pathway is an important negative modulator of allergic reactions.

RESULTS

Enhanced allergic responses in *Ptger3*$^{-/-}$ mice

Because studies have described attenuation by PGE_2 in various asthmatic responses[13,14], we subjected mice deficient in each individual prostaglandin E receptor subtype[15–17] to the ovalbumin (OVA)–induced asthma model and examined their responses. We backcrossed *Ptger1*$^{-/-}$, *Ptger2*$^{-/-}$ and *Ptger3*$^{-/-}$ mice for at least eight generations onto the C57BL/6 background and used wild-type C57BL/6 mice as controls. *Ptger4*$^{-/-}$ mice do not survive on the C57BL/6 background because of the development of patent ductus arteriosus[17,18]; we therefore intercrossed survivors of F_2 progeny and studied the

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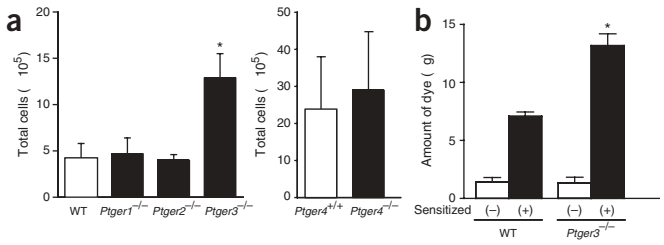

Figure 1 Enhanced allergic responses in *Ptger3*$^{-/-}$ mice. (**a**) Enhanced airway inflammation in *Ptger3*$^{-/-}$ mice. Wild-type (WT), *Ptger1*$^{-/-}$, *Ptger2*$^{-/-}$, *Ptger3*$^{-/-}$, *Ptger4*$^{+/+}$ and *Ptger4*$^{-/-}$ mice were immunized and were challenged with OVA. The total number of cells in BALF collected 24 h after the last OVA inhalation was determined. Data are means ± s.e.m. of values from six mice of each genotype. *, $P < 0.05$, versus wild-type, *Ptger1*$^{-/-}$ or *Ptger2*$^{-/-}$ mice. (**b**) Enhanced exudation reaction in the PCA test in *Ptger3*$^{-/-}$ mice. Data are means ± s.e.m. ($n = 5$). *, $P < 0.05$, versus sensitized wild-type mice. Data are representative of at least three independent experiments.

resultant *Ptger4*$^{+/+}$ and *Ptger4*$^{-/-}$ survivors on the mixed genetic background of 129/Ola × C57BL/6.

We used OVA-induced allergic asthma as a model of type I allergy[9]. We sensitized the mice by intraperitoneal injection of OVA on days 0 and 12 and then exposed them to aerosolized OVA on days 22, 26 and 30. We then killed the mice 24 h after the last antigen challenge and collected bronchoalveolar lavage fluid (BALF). Antigen inhalation induced an increase in the number of inflammatory cells in BALF in all strains of mice examined. However, the BALF of *Ptger3*$^{-/-}$ mice contained significantly more cells than that of wild-type, *Ptger1*$^{-/-}$ or *Ptger2*$^{-/-}$ mice (**Fig. 1a**). The number of cells in the BALF of *Ptger4*$^{-/-}$ mice was greater than that in wild-type C57BL/6 mice but did not differ significantly from that of *Ptger4*$^{+/+}$ mice (**Fig. 1a**).

We next examined whether the exaggerated allergic inflammation in *Ptger3*$^{-/-}$ mice could be attributed to a higher serum concentration of IgE. On day 12, after the second OVA injection, the serum concentrations of total IgE were 1,469 ± 676 pg/ml and 1,802 ± 325 pg/ml and those of OVA-specific IgE were 149 ± 85 U/ml and 112 ± 40 U/ml in wild-type and *Ptger3*$^{-/-}$ mice, respectively ($n = 5$ of each). On day 31, the concentration of total IgE in *Ptger3*$^{-/-}$ mice was significantly higher than that of wild-type mice (1,706 ± 542 pg/ml versus 592 ± 286 pg/ml; $n = 5$; $P < 0.05$). However, the concentration of OVA-specific IgE did not differ significantly between the two groups (41 ± 25 U/ml versus 72 ± 28 U/ml for wild-type and *Ptger3*$^{-/-}$ mice, respectively; $n = 5$; $P = 0.28$). *Ptger3*$^{-/-}$ mice thus demonstrated exaggerated allergic inflammation, as assessed by cellular infiltration, despite serum concentrations of antigen-specific IgE similar to those of wild-type mice, indicating that EP3 deficiency enhanced the allergic response at a step or steps subsequent to IgE synthesis. We confirmed this conclusion using a passive cutaneous anaphylaxis (PCA) test, which monitors the allergic response to fixed amounts of exogenous dinitrophenyl and dinitrophenyl-specific IgE. The exudation reaction of *Ptger3*$^{-/-}$ mice from the PCA test was significantly greater than that of wild-type mice in this assay (**Fig. 1b**).

Allergic inflammation is characterized by infiltration of eosinophilic leukocytes and T helper type 2 lymphocytes[3–5]. Eosinophils constituted the main fraction of the inflammatory cells in BALF of both *Ptger3*$^{-/-}$ and wild-type mice subjected to OVA sensitization and challenge. However, their number was significantly higher in *Ptger3*$^{-/-}$ mice than in wild-type mice (**Fig. 2a**). *Ptger3*$^{-/-}$ mice also had more lymphocytes and neutrophils in BALF, whereas the numbers of macrophages were similar for the two groups. Concentrations of the T helper type 2 cytokines interleukins 4, 5 and 13 in BALF were increased by antigen challenge in both groups (**Fig. 2b**). However, *Ptger3*$^{-/-}$ mice had much higher concentrations than did wild-type mice, whereas the concentration of the T helper type 1 cytokine interferon-γ in BALF did not differ significantly between the two genotypes ($P = 0.103$, wild-type versus *Ptger3*$^{-/-}$). Histological examination showed that lymphocytes, eosinophils and other leukocytes accumulated in the perivascular and peribronchial regions of the lungs of both groups of mice, but quantitative analysis showed that the cell infiltration was more extensive in *Ptger3*$^{-/-}$ mice (**Fig. 2c**).

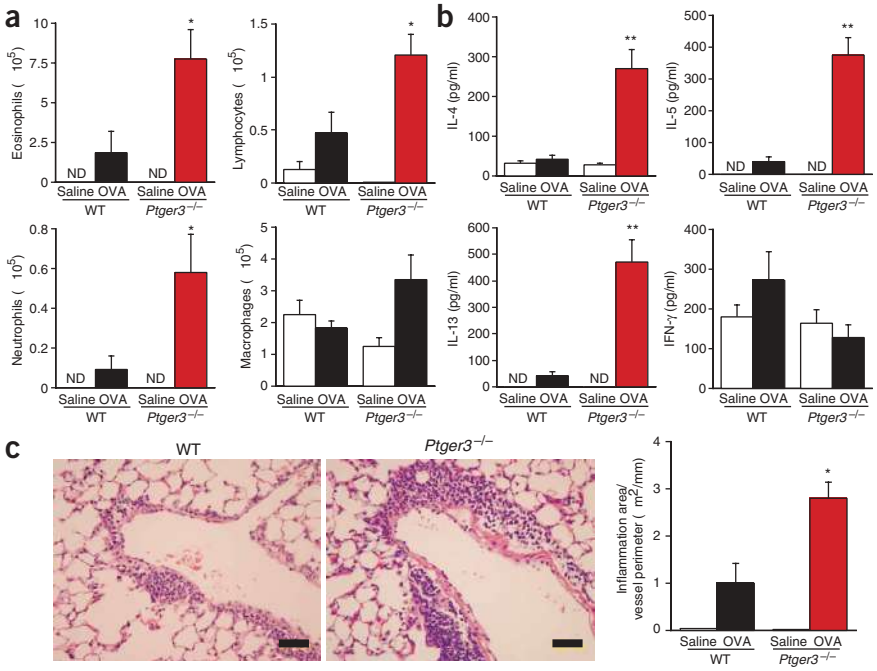

Figure 2 Enhanced inflammatory cell infiltration in the lungs of *Ptger3*$^{-/-}$ mice. (**a**) Cell population analysis. Eosinophils, lymphocytes, neutrophils, and macrophages were counted in BALF collected from OVA-sensitized wild-type or *Ptger3*$^{-/-}$ mice 24 h after the last inhalation of OVA or saline. Data are means ± s.e.m. ($n = 6$). (**b**) Cytokine measurement. ELISA of interleukin 4 (IL-4), IL-5, IL-13 and interferon-γ (IFN-γ) in BALF collected 8 h after the last inhalation as described in **a**. Data are means ± s.e.m. ($n = 6$). (**c**) Lung histology. The lungs of OVA-sensitized wild-type or *Ptger3*$^{-/-}$ mice were dissected 24 h after the last inhalation of OVA and were stained with hematoxylin and eosin (left). Scale bars, 50 μm. The area of inflammatory cell infiltration around pulmonary arterioles was quantified in four mice of each group (right). *, $P < 0.05$ and **, $P < 0.005$, versus OVA-challenged wild-type mice. ND, not detected. Data are representative of at least three independent experiments.

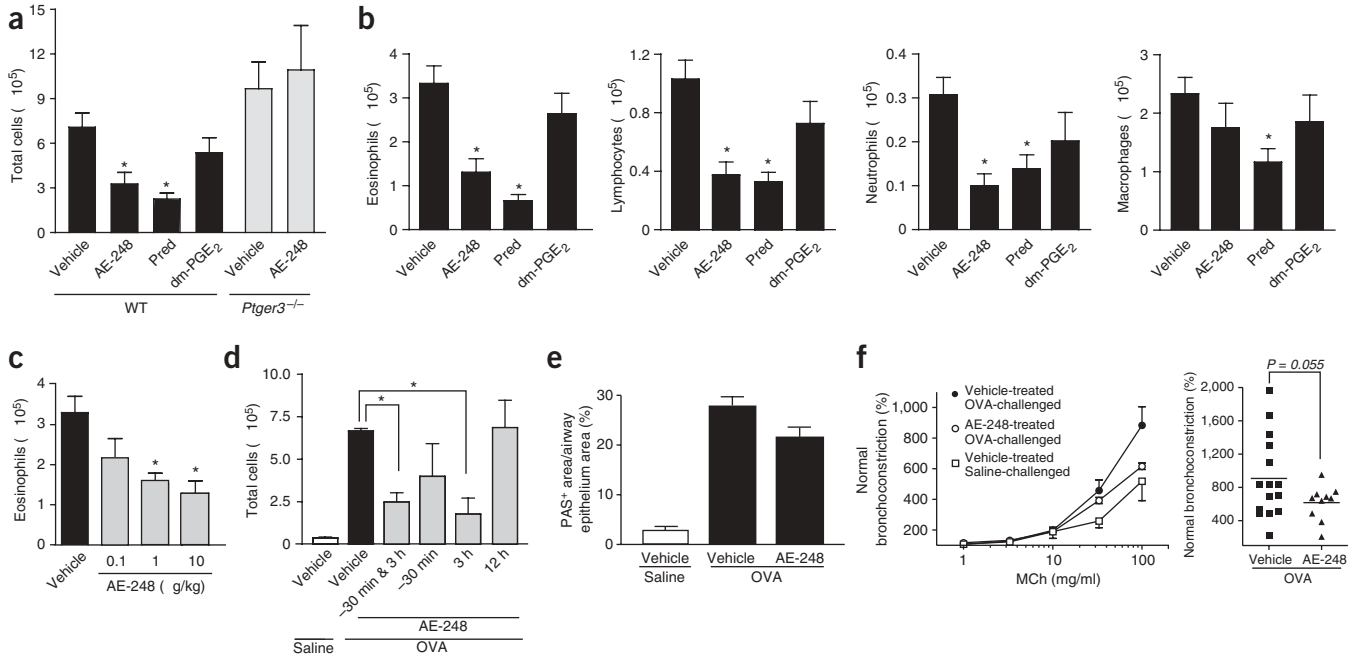

Figure 3 Suppression of airway inflammation by an EP3 agonist. (**a**,**b**) Effects of an EP3 agonist (AE-248; 10 μg/kg), prednisolone (Pred; 5 mg/kg) and dm-PGE_2 (10 μg/kg) on inflammatory cell infiltration in the lungs. Data represent total number of cells (**a**) and numbers of cell subsets (**b**) in BALF and are means ± s.e.m. ($n = 4$–8). (**c**) Dose-dependent effect of the EP3 agonist. AE-248 was administered at various doses to wild-type mice ($n = 7$–21) and the eosinophils in BALF were counted. (**d**) Effect of time of administration of the EP3 agonist. AE-248 was administered to wild-type mice ($n = 6$–8) on days 22, 26 and 30 either twice at 30 min before and 3 h after OVA challenge (−30 min & 3 h), once at 30 min before (−30 min) or once at 3 h or 12 h after the challenge. (**e**,**f**) Effects of the EP3 agonist on mucus secretion (**e**) and airway reactivity (**f**). Wild-type mice were sensitized with OVA and were treated with AE-248 or vehicle 3 h after each challenge with OVA or saline. Data represent the proportion of PAS^+ cells in the airway epithelium (**e**) or assessment of methacholine (MCh)–induced bronchoconstriction (**f**) 24 h after the last antigen challenge. (**f**) Dose-response curves (left) and responses to the maximum dose of Mch (100 mg/ml; right). Each symbol represents a different mouse. Data are means ± s.e.m. ($n = 6$–16). *, $P < 0.05$, versus vehicle-treated, OVA-challenged. Data are representative of or means ± s.e.m of three to five independent experiments.

Thus, the allergic inflammation in *Ptger3*$^{-/-}$ mice was much more pronounced than that in wild-type mice.

Antiallergic effects of an EP3 agonist *in vivo*

Our results suggested that PGE_2 acts at the EP3 receptor during elicitation of allergy and downregulates allergic reactions. To verify this conclusion, we examined the effects of an EP3-selective agonist, ONO-AE-248 (AE-248)[19], and of a nonselective stable PGE_2 analog, 16,16-dimethyl-PGE_2 (dm-PGE_2), on OVA-induced asthma in wild-type mice. We administered these compounds subcutaneously to OVA-sensitized mice beginning on day 21. As a positive control, we administered the glucocorticoid prednisolone, the drug used most prevalently for clinical asthma therapy, once a day from day 21 to day 30. We collected BALF 24 h after the last OVA challenge and assessed the effects of these compounds on cell infiltration. The EP3 agonist AE-248 significantly inhibited the antigen-induced infiltration of inflammatory cells (**Fig. 3a**). This inhibitory effect was absent from *Ptger3*$^{-/-}$ mice, confirming that it was mediated by EP3. In contrast, dm-PGE_2 did not significantly affect cell infiltration compared with that resulting from vehicle treatment at this dose ($P = 0.219$). The inhibitory effect of AE-248 at a dose of 10 μg/kg was similar to that obtained with prednisolone at a dose of 5 mg/kg for reducing asthmatic symptoms. Cell population analysis showed that the EP3 agonist significantly suppressed infiltration of eosinophils, lymphocytes and neutrophils to an extent similar to that noted with prednisolone; although prednisolone also inhibited macrophage infiltration, AE-248 did not (**Fig. 3b**). The suppressive effect of AE-248 on eosinophil infiltration was apparent at a dose of 0.1 μg/kg and was significant at doses of 1 μg/kg and 10 μg/kg (**Fig. 3c**).

We next attempted to optimize the regimen of AE-248 treatment with regard to suppression of the allergic reaction. We limited administration of the compound to the days of antigen challenge only (days 22, 26 and 30), either twice at 30 min before and 3 h after OVA challenge or once at either 30 min before or 3 or 12 h after the challenge. We compared the antiallergic effects at 24 h after the last antigen inhalation. We noted suppression of inflammatory cell infiltration similar to that obtained in the original treatment protocol in mice treated with the EP3 agonist either twice at 30 min before and 3 h after or once 3 h after antigen challenge on days 22, 26 and 30 only (**Fig. 3d**). Treatment with the compound once at 30 min before antigen challenge tended to reduce the number of inflammatory cells ($P = 0.165$, 30 min before versus vehicle), but treatment 12 h after challenge had no effect. In addition to the anti-inflammatory effect, administration of the EP3 agonist 3 h after OVA challenge tended to reduce the number of periodic acid Schiff (PAS)–positive cells in the lungs and airway hypersensitivity (**Fig. 3e**,**f**). These results indicate that administration of the EP3 agonist suppresses allergic inflammation.

EP3 agonist suppresses allergen-induced mediator release

Our results showed that PGE_2 acts at EP3 during allergy to suppress allergic inflammation. To provide mechanistic insight into this EP3-mediated action, we examined the effects of the EP3 agonist on antigen-induced allergic mediator release *in vitro*. We isolated cells

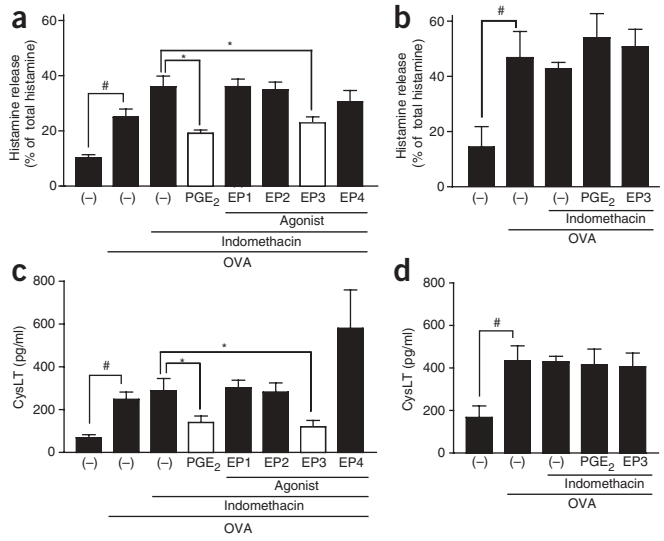

Figure 4 Inhibition by the EP3 agonist of antigen-induced mediator release. (**a**,**b**) Pieces of lung tissue obtained on day 19 from OVA-sensitized wild-type (**a**) or *Ptger3*$^{-/-}$ (**b**) mice were challenged *in vitro* with OVA in the absence (–) or presence of indomethacin or PGE_2 or the prostaglandin E receptor–selective agonists ONO-DI-004 (EP1), ONO-AE1-259 (EP2), ONO-AE-248 (EP3) or ONO-AE1-329 (EP4). Histamine release was then measured. (**c**,**d**) Lung tissue from wild-type (**c**) or *Ptger3*$^{-/-}$ (**d**) mice was incubated as described for **a** and **b**, after which cysteinyl leukotriene (CysLT) release was measured. Data are means ± s.e.m. ($n = 8$–10). #, $P < 0.05$, versus vehicle-treated; *, $P < 0.05$, versus indomethacin-treated, OVA-challenged lung. Data are means ± s.e.m of at least three independent experiments.

from the lungs from OVA-sensitized wild-type mice on day 19 after sensitization, which we then challenged by adding OVA to the cell cultures, and then measured the release of histamine and cysteinyl leukotrienes. The OVA challenge induced a significant increase in histamine release from the sensitized lung tissue cells (**Fig. 4a**). This effect of OVA was augmented by pretreatment of the tissue with indomethacin, and this effect of indomethacin was blocked by PGE_2 or AE-248 but not by agonists selective for EP1, EP2 or EP4.

To confirm the function of EP3 in this phenomenon, we repeated the experiments with lung tissue from OVA-sensitized *Ptger3*$^{-/-}$ mice. Stimulation of the tissue with OVA alone induced the release of a substantially more histamine than that found in wild-type mice. Moreover, this response was not affected by indomethacin, PGE_2 or AE-248 (**Fig. 4b**). We obtained essentially identical results by analysis of OVA-induced cysteinyl leukotriene release from lung tissue of wild-type and *Ptger3*$^{-/-}$ mice (**Fig. 4c**,**d**). Given that most of the histamine and cysteinyl leukotrienes released in response to antigen challenge is derived from mast cells, these results suggest that PGE_2 acts at EP3 in mast cells to suppress mediator release. The augmentation of mediator release by indomethacin further indicates that such inhibition occurs in a feedback way through endogenous PGE_2 production.

EP3 agonist inhibits allergy-related gene expression

Although our results showed that the EP3 agonist inhibits mediator release, this effect alone cannot explain the action of the compound *in vivo*, given that the agonist suppressed allergic inflammation even when administered 3 h after antigen inhalation (**Fig. 3d**). To further address the mechanism of action of the EP3 agonist, we did DNA microarray analysis. We subjected OVA-sensitized wild-type mice ($n = 3$ per group) to inhalation of either OVA or saline on days 22, 26 and 30 and injected the OVA-challenged mice with either vehicle or the EP3 agonist 3 h after each antigen challenge; we injected the saline-challenged control mice with vehicle instead of the EP3 agonist. We isolated lung tissue from all mice 24 h after the last inhalation and subjected it to microarray analysis with the MG-U74Av2 gene chip that includes probes for 12,426 mouse genes.

We then compared expression of each gene in each of the three control mice with that in each of the three vehicle-treated, OVA-challenged mice using Affymetrix GeneChip Expression Analysis software. We considered a change in the expression of a gene relevant if it differed in more than six of the nine pairs. The expression of 279 of the 12,426 genes was increased considerably and that of 86 genes was decreased considerably in the lungs of the OVA-challenged mice compared with that in the lungs of control mice (**Fig. 5a**). We then compared gene expression in the EP3 agonist–treated, OVA-challenged mice with that in the vehicle-treated, OVA-challenged mice in a similar way. Of the 279 genes that showed an antigen-induced increase in expression, 195 genes showed a decrease in expression in response to the EP3 agonist in more than six of the nine comparison pairs. The expression of the remaining 84 genes was also decreased by the agonist but in fewer than six of the nine pairs. Similarly, of the 86 genes that showed an antigen-induced decrease in expression, 38 genes showed an increase in expression in response to the EP3 agonist in more than six of the nine pairs (**Supplementary Table 1** online).

Among the genes upregulated by antigen challenge were those for immunoglobulins (68 of 279) and for receptors or markers of lymphocytes and other immune cells (18 of 279). The genes for chemokines or chemokine receptors accounted for 19 of these 279 genes. The upregulation of the first two groups of genes may represent recruitment of lymphocytes and other inflammatory cells, and the induction of the last group of chemokine and chemokine receptor genes may be responsible for such recruitment. The last group included genes encoding CCL17 (TARC), CCL11 (eotaxin) and CCL7 (MCP-3), the expression of each of which was upregulated significantly after antigen challenge and whose induction was completely inhibited by the EP3 agonist. We confirmed these changes by RT-PCR analysis (**Fig. 5b**). Each of these chemokines has been linked to allergic asthma and allergic rhinoconjunctivitis[4,5]. These results suggest that the EP3 agonist suppresses allergic inflammation through inhibition of the induction of chemokine genes.

We also detected induction by antigen exposure and suppression by the EP3 agonist of the expression of other genes involved in tissue metaplasia, remodeling and resolution[20–24], including those encoding Clca3 (also called Gob-5), Muc5ac, MMP-12, ADAM-8 and Alox15 (**Supplementary Table 1** online). Muc5ac encodes a core protein of large mucin, and Gob-5 is involved in mucus secretion in the epithelium[20]. Although the decrease in the number of PAS-positive cells by EP3 agonist was limited (**Fig. 3e**), suppression of induction of the genes encoding Gob-5 and Muc5ac indicates that the EP3 agonist treatment substantially attenuates goblet cell formation. In contrast, although transcripts of the genes encoding enzymes that contribute to PGE_2 biosynthesis, including cyclooxygenases 1 and 2 and microsomal prostaglandin E synthases 1 and 2, were present in normal lung tissue, their abundance was not substantially affected by OVA challenge with or without treatment with the EP3 agonist. Among the prostaglandin E receptor subtypes, expression of only the gene encoding EP4 was increased substantially by OVA challenge in a way that was sensitive to the EP3 agonist. These results indicate that EP3 agonist administered 3 h after the challenge inhibits allergen-induced gene expression changes.

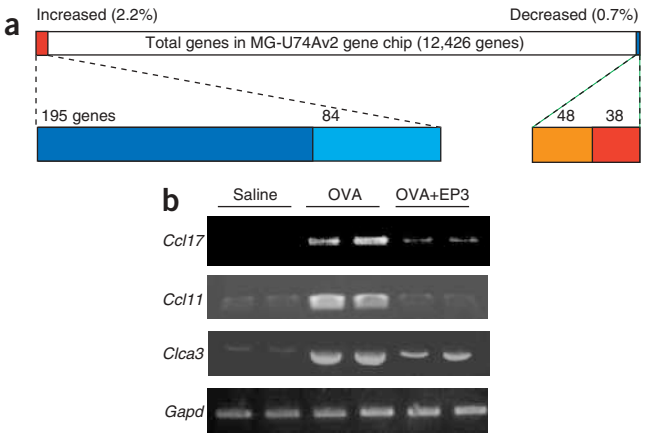

Figure 5 Effects of OVA challenge and the EP3 agonist on gene expression in the lungs. (**a**) Microarray analysis. Wild-type mice were sensitized with OVA and were treated with either AE-248 or vehicle 3 h after each challenge with OVA or saline. Lung tissue was isolated 24 h after the last challenge, and polyadenylated RNA was assessed by microarray analysis. Top bar, proportion of genes whose expression was increased (red) or decreased (blue) in the vehicle-treated, OVA-challenged mice compared with that in the vehicle-treated, saline-challenged mice. Bottom bars, effects of the EP3 agonist on the expression of genes affected by OVA challenge. Blue or turquoise, number of genes that decreased in more or less, respectively, than six of nine pairs by EP3 agonist treatment; red or orange, number of genes that increased in more or less, respectively, than six of nine pairs by EP3 agonist treatment. (**b**) RT-PCR analysis of the expression of *Ccl17* (TARC), *Ccl11* (eotaxin), *Clca3* (Gob-5) and *Gapd* (glyceraldehyde phosphodehydrogenase; loading control) in the lungs of vehicle-treated, saline-challenged (Saline), vehicle-treated, OVA-challenged (OVA) or EP3 agonist–treated, OVA-challenged (OVA+EP3) mice. Each lane represents one mouse. Data are representative of two or three experiments.

Localization of EP3 in the lungs

We next examined the localization of EP3 in the lungs. EP3 mRNA was detected in the lungs of wild-type mice by RT-PCR analysis, and its abundance was not affected by OVA challenge (**Fig. 6a**). Consistently, when we enriched BALF from challenged mice for $CD11b^+$ cells, including macrophages, neutrophils and eosinophils, or $CD4^+$ T cells, and analyzed the cells by RT-PCR, we detected little expression of EP3 mRNA in these cells (**Fig. 6a**). Given that antibodies to mouse EP3 suitable for immunohistochemistry were not available, we used *Ptger3*$^{-/-}$ mice in which the β-galactosidase gene was 'knocked-in' at the EP3 gene locus to examine EP3 localization. Staining for β-galactosidase activity showed positive signals in airway epithelial cells of the engineered mice but not in those of wild-type mice (**Fig. 6b**). In contrast, consistent with the PCR analysis reported above, β-galactosidase signals were barely detectable in the cells infiltrating in the lung parenchyma (**Fig. 6b**). To determine the possible relationship between EP3 localization and its regulation of gene expression, we examined by immunohistochemistry the site of allergen-induced chemokine production. CCL17 immunoreactivity was induced by OVA challenge in the airway epithelial cells of sensitized wild-type mice, and this effect was inhibited by treatment with the EP3 agonist (**Fig. 6c**). We obtained essentially identical results for CCL11 expression (data not shown). These data suggested that EP3 is constitutively present on the surface of airway epithelial cells and that EP3 signaling in these cells inhibits the allergen-induced expression of chemokine genes.

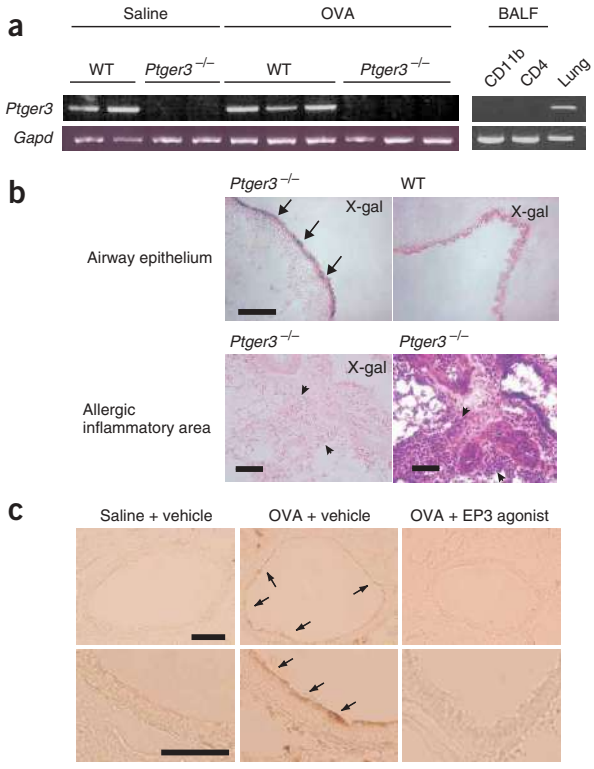

Figure 6 Localization of EP3 and CCL17 in the lungs. (**a**) RT-PCR analysis of EP3 (*Ptger3*) mRNA in lungs isolated from OVA-sensitized wild-type or *Ptger3*$^{-/-}$ mice 24 h after the last challenge with either saline or OVA (left) and in $CD11b^+$ cells and $CD4^+$ cells from BALF (right). Each lane represents one mouse. *Gapd*, loading control. (**b**) Histochemical staining for EP3 (X-gal). Lung tissue from *Ptger3*$^{-/-}$ mice expressing the β-galactosidase gene at the *Ptger3* locus was stained for β-galactosidase activity with the substrate X-gal. Arrows (top left) indicate positive signals (blue) on airway epithelial cells. Arrowheads (bottom row) indicate accumulation of inflammatory cells. Sections were counterstained with eosin (red). A section sequential to the X-gal staining (bottom left) was stained with hematoxylin and eosin (bottom right). Scale bars, 50 μm. (**c**) OVA-induced expression of CCL17 in airway epithelial cells and its suppression by the EP3 agonist. The lungs of OVA-sensitized wild-type mice were isolated 24 h after the last challenge with saline or OVA; the mice were also treated with AE-248 or vehicle 3 h after each challenge. The tissue was then immunostained for CCL17. Arrows indicate positive signals. Scale bars, 50 μm. Data are representative of at least three experiments.

DISCUSSION

Allergic inflammation is initiated by mast cell activation as acute vascular responses and develops into inflammation characterized by infiltration of T helper type 2 lymphocytes and eosinophils, leading to tissue metaplasia and remodeling. This process of progression is similarly seen in bronchial asthma and allergic rhinitis and conjunctivitis[3–5]. Using OVA–induced allergic asthma as a model, we have shown here that PGE_2-EP3 signaling negatively regulates this progression of allergic inflammation. This is consistent with published findings that inhaled PGE_2 suppresses allergen-induced inflammation in human asthmatic patients[13,25]. Given the similarity of allergic rhinoconjunctivitis to asthma, it is very likely that EP3 signaling also modulates the process of allergic rhinitis and allergic conjunctivitis.

The PGD_2–prostaglandin D receptor pathway mediates allergic responses[9]. That pathway and the PGE_2-EP3 pathway thus exert opposing actions in allergic inflammation. Furthermore, mice deficient

in the PGI_2 receptor have higher total and antigen-specific IgE concentrations than those in wild-type mice and manifest augmented asthmatic responses[26]. Cyclooxygenase metabolites and their cognate receptors thus participate in various steps of allergic asthma, with the PGD_2–prostaglandin D receptor pathway and the PGE_2-EP3 pathway functioning locally at the site of allergy to mediate and to suppress allergic inflammation, respectively, and the PGI_2 receptor pathway contributing to the primary immune response for IgE production. PGD_2 is produced in substantial amounts by activated mast cells, and our preliminary immunohistochemical study showed that microsomal prostaglandin E synthase 1 is highly expressed in the airway epithelial cells of antigen-challenged mice. These observations suggest that the general ineffectiveness of NSAIDs in allergic diseases might be due to inhibition of all of these pathways. Expression in the lung of the gene encoding arachidonate 15-lipoxygenase was enhanced considerably after OVA challenge and was suppressed by the treatment with the EP3 agonist (**Supplementary Table 1** online). Arachidonate 15-lipoxygenase is involved in formation of lipoxins, which are suggested to work in diminution of various forms of inflammation in the airway and gastrointestinal tract[24,27–29]. Because leukocytes are chief sources of this enzyme, a decrease in the expression of this gene by the EP3 agonist may be due to inhibition of recruitment of leukocytes to the lung. Thus, various eicosanoids work as anti-inflammatory messengers in different phases of inflammation.

The aggravation of allergic diseases by aspirin-like NSAIDs is known as aspirin intolerance and occurs in patients with asthma and/or nasal polyposis[11,12]. Despite a high incidence of aspirin intolerance in these patients, the underlying mechanism has remained controversial. A prevailing hypothesis has been that NSAIDs shunt the substrate arachidonic acid from the cyclooxygenase pathway to the alternative lipoxygenase pathway, resulting in enhanced production of proinflammatory and proasthmatic leukotrienes. Our finding that allergic reactions are enhanced in *Ptger3*$^{-/-}$ mice without inhibition of cyclooxygenase challenges this hypothesis and suggests that the PGE_2-EP3–mediated suppression of allergic inflammation is a long-sought target mechanism of aspirin intolerance. Although it remains unclear why patients with aspirin-induced asthma are more vulnerable to inhibition of prostaglandin synthesis, it is likely that these people depend more on protection by the PGE_2-EP3 pathway than do patients without aspirin-induced asthma.

We have shown that endogenous PGE_2 acting at EP3 has an important feedback function in allergic inflammation and that administration of an EP3-selective agonist substantially suppresses elicitation of allergic inflammatory reactions. Notably, the EP3 agonist was most effective in suppressing allergic inflammation when administered 3 h after antigen challenge. Allergic inflammation consists of two phases: the early-phase reaction, which develops and subsides within 60 min, and the late phase reaction, which starts at 3–6 h, peaks at 6–8 h and subsides 24 h after the antigen challenge. Local synthesis of chemokines and cytokines is crucial for the late-phase reaction, and the main site of their synthesis is the epithelium[3–5]. Notably, EP3 is expressed in airway epithelial cells, where the chemokines CCL11 and CCL17 are also expressed, and administration of the EP3 agonist 3 h after the challenge can inhibit the allergen-induced synthesis of these chemokines. A variety of mediators released from activated mast cells can induce expression of the genes encoding chemokines in the epithelium[4,5,30–32], and gene expression is detectable 3 h after antigen inhalation[33]. In our preliminary study, we found that the EP3 agonist administered 2 or 3 h after the antigen challenge was most effective, whereas agonist administered 1 or 4 h after the challenge was less effective in suppressing allergic inflammation. The time when treatment with the EP3 agonist is most effective thus coincides with the time of chemokine gene expression. These observations collectively suggest that PGE_2 acts at EP3 on airway epithelial cells and regulates the extent of the late-phase response by attenuating the expression of these chemokine genes. Notably, the prostaglandin D receptor is also expressed in airway epithelial cells, where it acts to enhance allergic inflammation[9].

Ptger3$^{-/-}$ mice demonstrate not only exaggerated asthmatic reactions but also enhancement of the PCA reaction, which is caused directly by allergic mediators released from mast cells. Consistent with that observation, we found that the EP3 agonist significantly suppressed the release of histamine and cysteinyl leukotrienes from antigen-sensitized lung tissue. The effects of PGE_2 on the release of mediators from mast cells have remained unclear. Mast cells derived from the bone marrow of mice deficient in EP3 have demonstrated the importance of PGE_2-EP3 signaling in potentiating antigen-stimulated degranulation[34]. We have detected the EP3 expression in bone marrow–derived mast cells from wild-type mice and found that administration of the EP3 agonist indeed augmented the antigen-induced histamine release (data not shown). In addition, PGE_2 and prostaglandin E analogs were shown to enhance mediator release by an EP1- and/or EP3-dependent mechanism in the MC-9 mast cell line[35]. In contrast, another study showed that the EP2 and EP3 agonist misoprostol inhibited mediator release from rat peritoneal mast cells triggered by antibody to IgE[36]. Given the existence of several subclasses of mast cells that differ in their origin and functions[37], these different responses to PGE_2 might reflect the differences in cell subclass. Bone marrow–derived mast cells are considered the presumptive *in vitro* counterpart of *in vivo*–differentiated mucosal mast cells and are different from mast cells working in allergic reactions in the lung[38]. Our findings suggest that a subclass of mast cells sensitive to EP3 stimulation has a chief function in OVA-induced allergic responses. Alternatively, an EP3-dependent indirect mechanism may operate to suppress mast cell activation in sensitized lungs *in vivo*.

The EP3 agonist is as potent as the glucocorticoid prednisolone in suppressing allergic inflammation and is able to exert this inhibitory effect at doses in the mg/kg range. Signaling by EP3 is mediated mainly by coupling to the inhibitory G protein G_i and results in inhibition of the generation of cyclic AMP. This signaling is elicited at PGE_2 or EP3 agonist concentrations as low as 10 pM (ref. 39). The antiallergic effect of low doses of the EP3 agonist in the present study might therefore be mediated by this signaling pathway. In addition inhibiting cyclic AMP production, PGE_2 acting at EP3 induces an increase in the intracellular free calcium concentration at doses of 10 nM to 1 μM, an effect mediating such EP3 actions as uterine contraction. Given these facts and also given that the proinflammatory action of PGE_2 is exerted by the EP2 and/or EP4 receptor, the EP3 agonist can circumvent the proinflammatory effects of PGE_2 by its selectivity and also its own adverse effects by administration of a low dose. Its unique property of being effective when administered after antigen challenge, that is, after appearance of early signs of allergy, may make an EP3 agonist a candidate for a new type of antiallergy drug.

METHODS

Animals and compounds. *Ptger1*$^{-/-}$, *Ptger2*$^{-/-}$, *Ptger3*$^{-/-}$ and *Ptger4*$^{-/-}$ mice were generated as described[15–17]. Mice were maintained on a 12-hour–12-hour light–dark cycle in specific pathogen–free conditions. All experimental procedures were approved by the Committee on Animal Research of Kyoto University Faculty of Medicine (Kyoto, Japan). The agonists ONO-DI-004, ONO-AE1-259, ONO-AE-248 and ONO-AE1-329 were supplied by ONO Pharmaceutical; the ligand-binding specificities of these compounds for each

prostaglandin E receptor subtype have been described[16]. We assessed the pharmacokinetic properties of AE-248 by monitoring the biological response to this compound administered intravenously; injection of rats with a bolus of AE-248 at a dose of 10 μg/kg resulted an increase in blood pressure of in 10 mmHg that persisted for 10 min (data not shown).

Analysis of asthmatic responses. Mice were immunized with OVA and were challenged by OVA inhalation as described[9]. BALF was collected at 8 and 24 h for cytokine measurement and cell analysis, respectively. The concentration of cytokines was measured using enzyme-linked immunosorbent assay (ELISA) kits (Endogen). The number of white blood cells was determined with a hemocytometer, and differential leukocyte counts were made using a smear stained with Diff-Quik solution (International Reagents). The serum concentrations of total and OVA-specific IgE were measured by sandwich ELISA as described[40].

For examination of the effects of AE-248, dm-PGE_2 (Sigma) and prednisolone (Sigma) on allergic inflammation in the lung, AE-248 (0.1–10 μg/kg) and dm-PGE_2 (10 μg/kg) were administered subcutaneously to OVA-sensitized mice twice a day beginning on day 21, the day before the first OVA challenge; they were also administered in an additional dose 3 h after antigen challenge on days 22, 26 and 30. Prednisolone (5 mg/kg, subcutaneously) was administered once a day from day 21 to day 30. For time course study, administration of the AE-248 was limited to the days of antigen challenge only (days 22, 26 and 30) and the following four treatment protocols were used: twice at 30 min before and 3 h after OVA challenge; once at 30 min before challenge; once at 3 h after the challenge; and once at 12 h after the challenge. BALF was collected 24 h after the last challenge and the accumulation of inflammatory cells was assessed.

For measurement of airway resistance, mice treated with the EP3 agonist or vehicle (saline) were anesthetized 24 h after the last antigen challenge and were ventilated at a tidal volume of 8 ml and a frequency of 2.5 Hz with a Flexivent SAV system (Scientific Respiratory Equipment) as described[41]. Each mouse was challenged by inhalation for 30 s of increasing doses of methacholine (1–100 mg/ml), with the doses administered at intervals of 5 min. Airway resistance was monitored with a heated pneumotachograph, and the peak value induced by each challenge was recorded using an oscillation technique.

Histological analysis. At 24 h after the last antigen challenge, lungs were distended by injection through the trachea of 10% formalin in 0.1 M sodium phosphate, pH 7.4, and then were excised. After immersion in fresh fixative for 24 h, the tissue was embedded in paraffin, sliced into sections 3 μm in thickness and stained with hematoxylin and eosin or with PAS solution. Quantitative analysis was done on microscopic images of three ×100 magnification fields per slide and three slides per mouse for each stain with the use of ImagePro Plus software (Media Cybernetics). Perivascular inflammatory cell infiltration was quantified as the total area of inflammatory cell accumulation relative to the perimeter of the blood vessel. For quantification of mucus-producing cells, the ratio of the PAS-positive area to the total area of the bronchial epithelium was determined. Slides were analyzed by investigators 'blinded' to sample identity and were presented in a random order for each examination.

PCA test. PCA was induced as described[42]. Mice were injected intradermally on the back with 20 μl of antibody to dinitrophenyl IgE (0.3 μg/ml). After 24 h, 0.25 mg of dinitrophenyl-conjugated bovine serum albumin and 1.25 mg of Evans blue in 0.25 ml of saline were injected intravenously to elicit PCA. Vascular permeability was evaluated by measurement of the amount of dye extravasated during the 30 min immediately after antigen challenge. Control mice were injected with the antigen and Evans blue without prior injection with IgE.

Histamine and cysteinyl leukotriene release *in vitro*. OVA-sensitized mice were killed by exsanguination on day 19 and the lungs were immediately removed and placed in Tyrode's solution. After removal of bronchial tissue and blood vessels, the lungs were chopped into fragments 0.5 mm^3 in size, and the fragments were washed and blotted dry. The tissue fragments (100 mg, wet weight) were suspended in Tyrode's solution at a final volume of 2 ml, were shaken for 10 min at 37 °C in the absence or presence of indomethacin (10 μM), PGE_2 (10 μM) or prostaglandin E receptor agonist (10 μM for EP1, EP2 and EP3 agonists; 1 μM for the EP4 agonist), and then were challenged for 15 min with OVA (10 μg/ml). The tissue and medium were separated by filtration and histamine was extracted from the tissue and the medium and assayed as described[43]. Histamine release is expressed as a percentage of total tissue histamine. Cysteinyl leukotrienes were extracted from the incubation medium with the use of a Sep-Pak C_{18} cartridge[44] and were assayed with an ELISA kit for LTC_4, LTD_4 and LTE_4 (Amersham Pharmacia Biotech).

DNA microarray analysis. At 3 h after each antigen challenge, OVA-challenged mice were injected with either vehicle or AE-248; saline-challenged control mice were injected with vehicle instead of the EP3 agonist. Lung tissue was isolated from all mice 24 h after the last inhalation. Polyadenylated RNA was prepared from lung tissue as described[8] and was subjected to microarray analysis with the MG-U74Av2 gene chip (Affymetrix). Expression of each gene in each of the three control mice was then compared with that in each of the three vehicle-treated, OVA-challenged mice using Affymetrix GeneChip Expression Analysis software (Suite, version 4.0, $3 \times 3 = 9$ comparison pairs). A change in the expression of a gene was considered relevant if it differed in more than six of the nine pairs. Comparison of gene expression in the EP3 agonist–treated, OVA-challenged mice with that in the vehicle-treated, OVA-challenged mice was done in a similar way.

RT-PCR. A GeneAmp PCR system 9700 (Perkin Elmer Applied Biosystems) was used for PCR. Primers (sense and antisense, respectively) were 5′-ATCCTCGTG TACCTGTCACAGCGACGCTGG-3′ and 5′-TGCTCAACCGACATCTGATT GAAGATCATT-3′ for *Ptger3* (EP3); 5′-CAGGAAGTTGGTGAGCTGGTATA-3′ and 5′-TTGTGTTCGCCTGTAGTGCATA-3′ for *Ccl17* (TARC); 5′-AGAGCTC CACAGCGCTTCTATT-3′ and 5′-GGTGCATCTGTTGTTGGTGATT-3′ for *Ccl11* (eotaxin); 5′-CACAACACTAAGGTGGCCTACCTCCAAG-3′ and 5′-AC TTGGAGTGAGGTGTTGAAGTGGTCCCT-3′ for *Clca3* (Gob-5); and 5′-TGA AGGTCGGTGTGAACGGATTTGGC-3′ and 5′-CATGTAGGCCATGAGGTCCA CCAC-3′ for *Gapd* (glyceraldehyde-3-phosphodehydrogenase).

Cell preparation. AutoMACS (Miltenyi Biotec) was used for preparation of the subpopulations of inflammatory cells in BALF. BALF was recovered 24 h after last challenge from OVA-challenged mice. Samples were enriched for $CD11b^+$ and $CD4^+$ cells by auto-MACS with antibody to CD11b or to CD4 MicroBeads, respectively (Miltenyi Biotec). Cells in each subpopulation were examined by smear staining using Diff-Quik solution.

Histochemistry. For staining to assess β-galactosidase activity, freshly isolated lung tissue was embedded in Tissue-Tec optimum cutting temperature compound (Sakura Finetechnicals), was frozen at −80 °C and was cut into sections 14 μm in thickness using a cryostat. The sections were fixed for 30 min at 4 °C with 2% paraformaldehyde in PBS, were washed with PBS and were incubated for 48 h at 30 °C with a solution containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; 1 mg/ml), 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 2 mM $MgCl_2$, 0.01% SDS and 0.02% Nonidet P-40. For immunohistochemistry, the lungs were first fixed by systemic perfusion with 4% paraformaldehyde in PBS and then were dissected. The dissected tissue was fixed further for 4 h and then was incubated overnight at 4 °C with 15% sucrose. Sections with a thickness of 14 μm were then prepared and, after inhibition of endogenous peroxidase activity with Peroxo-Block (Zymed), were incubated overnight at 4 °C with goat anti–mouse TARC (AF529; R&D Systems). Immune complexes were detected by incubation for 1 h at 25 °C with horseradish peroxidase–conjugated secondary antibodies (Zymed) followed by exposure to 3,3′-diaminobenzidine.

Statistical analysis. Data are presented as means ± s.e.m. and were analyzed by the unpaired *t*-test, Mann-Whitney test, or one-way or two-way analysis of variance followed by Dunnett's test with the use of Prism 3.0 and SPSS 10.1 software. A *P* value of less than 0.05 was considered statistically significant.

GEO accession number. Microarray data, GSE2276.

Note: Supplementary information is available on the Nature Immunology website.

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The authors declare competing financial interests (see the *Nature Immunology* website for details).

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1. Sears, M.R. Epidemiology of childhood asthma. *Lancet* **350**, 1015–1020 (1997).
2. Arshad, S.H. & Holgate, S. The role of IgE in allergen-induced inflammation and the potential for intervention with a humanized monoclonal anti-IgE antibody. *Clin. Exp. Allergy* **31**, 1344–1351 (2001).
3. Corry, D.B. & Kheradmand, F. Induction and regulation of the IgE response. *Nature* **402**, B18–B23 (1999).
4. Christodoulopoulos, P., Cameron, L., Durham, S. & Hamid, Q. Molecular pathology of allergic disease II: Upper airway disease. *J. Allergy Clin. Immunol.* **105**, 211–223 (2000).
5. Irkec, M. & Bozkurt, B. Epithelial cells in ocular allergy. *Curr. Allergy Asthma Rep.* **3**, 352–357 (2003).
6. Murray, J.J. *et al.* Release of prostaglandin D_2 into human airways during acute antigen challenge. *N. Engl. J. Med.* **315**, 800–804 (1986).
7. Howarth, P.H. Mediators of nasal blockage in allergic rhinitis. *Allergy* **52**, 12–18 (1997).
8. Narumiya, S., Sugimoto, Y. & Ushikubi, F. Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* **79**, 1193–1226 (1999).
9. Matsuoka, T. *et al.* Prostaglandin D_2 as a mediator of allergic asthma. *Science* **287**, 2013–2017 (2000).
10. Oguma, T. *et al.* Role of prostanoid DP receptor variants in susceptibility to asthma. *N. Engl. J. Med.* **351**, 1752–1763 (2004).
11. Szczeklik, A. & Stevenson, D.D. Aspirin-induced asthma: advances in pathogenesis and management. *J. Allergy Clin. Immunol.* **104**, 5–13 (1999).
12. Pawankar, R. Nasal polyposis: an update. *Curr. Opin. Allergy Clin. Immunol.* **3**, 1–6 (2003).
13. Gauvreau, G.M., Watson, R.M. & O'Byrne, P.M. Protective effects of inhaled PGE_2 on allergen-induced airway responses and airway inflammation. *Am. J. Respir. Crit. Care Med.* **159**, 31–36 (1999).
14. Martin, J.G. *et al.* The immunomodulatory actions of prostaglandin E_2 on allergic airway responses in the rat. *J. Immunol.* **169**, 3963–3969 (2002).
15. Ushikubi, F. *et al.* Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* **395**, 281–284 (1998).
16. Hizaki, H. *et al.* Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP2. *Proc. Natl. Acad. Sci. USA* **96**, 10501–10506 (1999).
17. Segi, E. *et al.* Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. *Biochem. Biophys. Res. Commun.* **246**, 7–12 (1998).
18. Nguyen, M. *et al.* The prostaglandin receptor EP4 triggers remodelling of the cardiovascular system at birth. *Nature* **390**, 78–81 (1997).
19. Suzawa, T. *et al.* The role of prostaglandin E receptor subtypes (EP1, EP2, EP3, and EP4) in bone resorption: an analysis using specific agonists for the respective EPs. *Endocrinology* **141**, 1554–1559 (2000).
20. Nakanishi, A. *et al.* Role of gob-5 in mucus overproduction and airway hyperresponsiveness in asthma. *Proc. Natl. Acad. Sci. USA* **98**, 5175–5180 (2001).
21. Zuhdi, A.M. *et al.* Muc-5/5ac mucin messenger RNA and protein expression is a marker of goblet cell metaplasia in murine airways. *Am. J. Respir. Cell Mol. Biol.* **22**, 253–260 (2000).
22. Pouladi, M.A. *et al.* Interleukin-13-dependent expression of matrix metalloproteinase-12 is required for the development of airway eosinophilia in mice. *Am. J. Respir. Cell Mol. Biol.* **30**, 84–90 (2004).
23. King, N.E. *et al.* Expression and regulation of a disintegrin and metalloproteinase (ADAM) 8 in experimental asthma. *Am. J. Respir. Cell Mol. Biol.* **31**, 257–265 (2004).
24. Levy, B.D. *et al.* Lipid mediator class switching during acute inflammation: signals in resolution. *Nat. Immunol.* **2**, 612–619 (2001).
25. Pavord, I.D., Wong, C.S., Williams, J. & Tattersfield, A.E. Effect of inhaled prostaglandin E_2 on allergen-induced asthma. *Am. Rev. Respir. Dis.* **148**, 87–90 (1993).
26. Takahashi, Y. *et al.* Augmentation of allergic inflammation in prostanoid IP receptor deficient mice. *Br. J. Pharmacol.* **137**, 315–322 (2002).
27. Levy, B.D. *et al.* Multi-pronged inhibition of airway hyper-responsiveness and inflammation by lipoxin A_4. *Nat. Med.* **8**, 1018–1023 (2002).
28. Karp, C.L. *et al.* Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway. *Nat. Immunol.* **5**, 388–392 (2004).
29. Fiorucci, S. *et al.* A β-oxidation-resistant lipoxin A_4 analog treats hapten-induced colitis by attenuating inflammation and immune dysfunction. *Proc. Natl. Acad. Sci. USA* **101**, 15736–15741 (2004).
30. Plaut, M. *et al.* Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophores. *Nature* **339**, 64–67 (1989).
31. Matsukura, S. *et al.* Interleukin-13 upregulates eotaxin expression in airway epithelial cells by a STAT6-dependent mechanism. *Am. J. Respir. Cell Mol. Biol.* **24**, 755–761 (2001).
32. Berin, M.C., Eckmann, L., Broide, D.H. & Kagnoff, M.F. Regulated production of the T helper 2-type T-cell chemoattractant TARC by human bronchial epithelial cells in vitro and in human lung xenografts. *Am. J. Respir. Cell Mol. Biol.* **24**, 382–389 (2001).
33. Kawasaki, S. *et al.* Intervention of thymus and activation-regulated chemokine attenuates the development of allergic airway inflammation and hyperresponsiveness in mice. *J. Immunol.* **166**, 2055–2062 (2001).
34. Nguyen, M. *et al.* Receptors and signaling mechanisms required for prostaglandin E_2-mediated regulation of mast cell degranulation and IL-6 production. *J. Immunol.* **169**, 4586–4593 (2002).
35. Gomi, K., Zhu, F.G. & Marshall, J.S. Prostaglandin E_2 selectively enhances the IgE-mediated production of IL-6 and granulocyte-macrophage colony-stimulating factor by mast cells through an EP1/EP3-dependent mechanism. *J. Immunol.* **165**, 6545–6552 (2000).
36. Chan, C.L., Jones, R.L. & Lau, H.Y. Characterization of prostanoid receptors mediating inhibition of histamine release from anti-IgE-activated rat peritoneal mast cells. *Br. J. Pharmacol.* **129**, 589–597 (2000).
37. Gurish, M.F. & Austen, K.F. The diverse roles of mast cells. *J. Exp. Med.* **194**, F1–F5 (2001).
38. Stevens, R.L., Rothenberg, M.E., Levi-Schaffer, F. & Austen, K.F. Ontogeny of in vitro-differentiated mouse mast cells. *Fed. Proc.* **46**, 1915–1919 (1987).
39. Sugimoto, Y. *et al.* Two isoforms of the EP3 receptor with different carboxyl-terminal domains. Identical ligand binding properties and different coupling properties with Gi proteins. *J. Biol. Chem.* **268**, 2712–2718 (1993).
40. Nagai, H. *et al.* Effect of overproduction of interleukin 5 on dinitrofluorobenzene-induced allergic cutaneous response in mice. *J. Pharmacol. Exp. Ther.* **288**, 43–50 (1999).
41. Gomes, R.F., Shen, X., Ramchandani, R., Tepper, R.S. & Bates, J.H. Comparative respiratory system mechanics in rodents. *J. Appl. Physiol.* **89**, 908–916 (2000).
42. Inagaki, N. *et al.* Participation of histamine H_1 and H_2 receptors in passive cutaneous anaphylaxis-induced scratching behavior in ICR mice. *Eur. J. Pharmacol.* **367**, 361–371 (1999).
43. Shore, P.A., Burkhalter, A. & Cohn, V.H., Jr. A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* **127**, 182–186 (1959).
44. Powell, W.S. Rapid extraction of arachidonic acid metabolites from biological samples using octadecylsilyl silica. *Methods Enzymol.* **86**, 467–477 (1982).