British Journal of Pharmacology (2002) 137, 315–322

www.nature.com/bjp

Augmentation of allergic inflammation in prostanoid IP receptor deficient mice

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1 To evaluate the role of prostaglandin I_2 (PGI_2) in allergic inflammation, allergic responses in the airway, skin and T cells were studied in mice lacking the receptor for PGI_2 (the prostanoid IP receptor) through gene disruption.

2 Three inhalations of antigen caused an increase in plasma extravasation, leukocyte accumulation and cytokine (interleukin (IL)-4 and IL-5) production in the airway of sensitized mice. These airway inflammatory responses were significantly greater in IP receptor deficient mice than in wild-type mice.

3 The vascular leakage caused by passive cutaneous anaphylaxis, substance P and 5-hydroxytryptamine was markedly increased in the skin of IP receptor deficient mice, compared with comparably treated wild-type mice.

4 The inhalation of antigen in sensitized mice resulted in increased serum antigen specific IgE, total IgE and IgG levels. The magnitude of the elevations of each immunoglobulin level in IP receptor deficient mice is notably higher than that in wild-type mice. To elucidate the mechanism of an enhancement of immunoglobulin production, the activity of T cells in sensitized and non-sensitized mice was studied by means of the production of cytokines. The antigen-induced IL-4 production by spleen cells from sensitized IP receptor deficient mice was almost three times greater than that in wild-type mice. On the contrary, the anti-CD3 antibody-induced interferon-γ production by $CD4^+$ T cells from non-sensitized IP receptor deficient mice was significantly lower than that in wild-type mice.

5 The present data indicate that IP receptor deficiency reinforced an allergic airway and skin inflammation by augmentation of vascular permeability increase and the T helper 2 cell function. These findings suggest a regulatory role of PGI_2 in allergic inflammation.
British Journal of Pharmacology (2002) **137,** 315–322. doi:10.1038/sj.bjp.0704872

Keywords: Allergic response; IP receptor; prostaglandin I_2; T cell function

Abbreviations: Ach, acetylcholine chloride; BALF, bronchoalveolar lavage fluid; BSA, bovine serum albumin; DNFB, dinitrofluorobenzene; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FBS, foetal bovine serum; IFN, interferon; IL, interleukin; OA, ovalbumin; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; PG, prostaglandin; Th, T helper; TNF, tumour necrosis factor

Introduction

Prostaglandins (PGs) are cyclo-oxygenase metabolites of arachidonic acid and are generated in response to various stimuli to cells. They are locally acting autacoids with pleiotropic roles in physiologic and pathophysiologic processes (Arias, 2000; Campbell & Halushka, 1996; Imig, 2000; Seibert *et al*., 1999). Under physiological conditions, they mainly work in the gastrointestinal tract, the circulatory system and regenerative organs. Moreover, PGs play a role in a variety of pathophysiological situations including inflammation (Ghosh *et al*., 2000; Romay *et al*., 2000; Murata *et al*., 1997) and allergic response.

As for the role of PGs in allergic response, Gavett *et al*. (1999) reported that allergic lung responses were enhanced in cyclo-oxygenase-deficient mice. They demonstrated exaggeration of lung eosinophilia, IgE production and airway hyperresponsiveness in cyclo-oxygenase-1 and cyclo-oxygenase-2 deficient mice. Their findings indicated that the inhibition of PG production resulted in an enhancement of allergic responses.

Concerning the role of individual PGs, the production of each PG in allergic lesions and the effect of extrinsic PGs on allergic responses were investigated. The release of many PGs has been demonstrated after allergic reaction in several cells or tissues (Dahlen *et al*., 1983; Lahti *et al*., 1983; Meijer *et al*., 1996; Montuschi *et al*., 1999; Oosterhoff *et al*., 1995; Schulman *et al*., 1981; Wang *et al*., 1995; Woodward *et al*., 1995; Yamasaki *et al*., 1997). Among PGs, the role of PGD_2 and PGE_2 was thoroughly investigated. In previous studies, we have demonstrated a role for PGD_2 in allergic asthmatic responses by studies in PGD_2 receptor gene-disrupted mice (Matsuoka *et al*., 2000). More recent studies also indicate that PGD_2 plays a role in T helper (Th) 2- and eosinophil-

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dependent inflammation (Hirai *et al*., 2001; Tanaka *et al*., 2000). In addition to PGD_2, the role of PGE_2 in allergic inflammation was also investigated (Gauvreau *et al*., 1999; Sestini *et al*., 1996; Szczeklik *et al*., 1996). Whereas PGE_2 showed an inhibition of allergic mediator release and antigen-induced broncoconstriction, it potentiated the *in vitro* Th2 response, e.g. IgE production (Betz & Fox, 1991; Gold *et al*., 1994; Hilkens *et al*., 1995; Kalinski *et al*., 1997; Paliogianni & Boumpas, 1996; Parker *et al*., 1995; van der Pouw Kraan *et al*., 1995). These results suggest that PGE_2 has an opposite pharmacological action in allergic inflammation.

In addition to the above two PGs, another PG, PGI_2, could be involved in allergic reactions. PGI_2 was produced by allergic reaction in human lung, human skin, guinea-pig lung and other tissues (Dahlen *et al*., 1983; Lahti *et al*., 1983; Schulman *et al*., 1981). PGI_2 also showed an inhibition of allergic mediator release and eosinophil recruitment in human and experimental animals (Burka & Garland, 1977; Lellouch-Tubiana *et al*., 1988; Wu *et al*., 1998). These findings suggest the participation of PGI_2 in allergic responses. Therefore, this study was conducted to clarify the role of PGI_2 in allergic responses by measuring those responses in mice with disruption of the gene for the PGI_2 receptor (IP receptor deficient mice).

Methods

Animals

Seven- to twelve-week-old male mice, with the gene for the IP receptor disrupted (129Sv × C57BL/6 background; backcross to C57BL/6 five times (N5)) weighing 21 – 30 g were obtained by the same method previously described (Matsuoka *et al*., 2000). Age- and generation-matched male wild-type animals were used as controls. The animals were housed in plastic cages in an air-conditioned room at $22 \pm 1^{\circ}C$ with a relative humidity of $60 \pm 5\%$, fed a standard laboratory diet and given water *ad libitum*. Experiments were performed following the guidelines for the care and use of experimental animals set by the Japanese Association for Laboratory Animals Science in 1987.

Agents

Ovalbumin (OA; Seikagaku Kogyo, Tokyo, Japan), bovine serum albumin (BSA; Seikagaku Kogyo), acetylcholine chloride (Ach; Nacalai Tesque, Kyoto, Japan), Türk solution (Wako Pure Chemical Industries Ltd., Osaka, Japan), pentobarbitone sodium (Abbott Laboratories, Chicago, IL, U.S.A.), disodium ethylenediaminetetraacetic acid (EDTA-2Na; Nacalai Tesque), Diff-Quick solution (International Reagent Corp., Kobe, Japan) were purchased.

Sensitization and antigen challenge for airway inflammation

Mice were actively sensitized by intraperitoneal injections of 50 μg OA with 1 mg alum on days 0 and 12. Starting on day 22, they were exposed to aerosolized OA (10 mg ml^{-1} in 0.9% NaCl solution) for 30 min, three times every fourth day (days 22, 26 and 30) according to the method reported by Nagai *et al*. (1996). The aerosol (particle size; 2.0 – 6.0 μm^3) was generated by a nebulizer (Ultrasonic nebulizer TN-701, Azwell, Osaka, Japan) driven by a vacuum pump. Mice were exposed to aerosolized antigen in a perspex cylinder chamber (diameter 5.5 cm, height 12 cm). Control wild-type animals were exposed to saline in a similar manner. Negative control animals were injected with saline and then exposed to saline.

Measurement of leukocyte number, albumin level and cytokine levels in bronchoalveolar lavage fluid (BALF)

The following experiments were carried out according to previously described methods (Nagai *et al*., 1996). The mice were sensitized and challenged with antigen by the methods described above. Eight hours after the last inhalation of antigen (30 days after the first immunization), the mice were killed with an intraperitoneal injection of sodium pentobarbitone (100 mg kg^{-1}). The trachea was cannulated and the airway lumen was washed four times with 1 ml of calcium- and magnesium-free phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 0.05 mM EDTA-2Na (total volume; 3 ml, recovery > 85%). Bronchoalveolar lavage fluid (BALF) from each animal was pooled in a plastic tube, cooled in ice, and centrifuged ($150 \times g$) at $4^{\circ}C$ for 10 min. Cell pellets were resuspended in the same buffer (1 ml). BALF was stained with Türk solution and the number of nucleated cells was counted in a chamber. A differential count was made on a smear prepared with a cytocentrifuge (Cytospine II, Shandon, U.K.) and stained with Diff-Quick solution (based on standard morphologic criteria) on at least 300 cells (magnification × 500). To measure the amount of albumin, interleukin (IL)-4 and IL-5, BALF was obtained by employing the same buffer described above (without BSA). Measurement was carried out using enzyme-linked immunosorbent assay (ELISA) (Funakoshi, Tokyo, Japan and Endogen Inc., Cambridge, MA, U.S.A.).

Histological study

To confirm the presence of airway inflammation, histopathological studies were carried out. The mice were killed 24 h after the third antigen inhalation, and the whole lungs were distended by instillation of 10% buffered formalin *via* the trachea, removed, and immersed in the same fixative with the trachea clamped for 24 h. The tissue was sliced and embedded in paraffin, and 6 μm sections were stained with haematoxylin and eosin for light microscopic examination.

Vascular leakage in the skin

Vascular leakage in the skin was caused by three different stimuli, passive cutaneous anaphylaxis (PCA), substance P and 5-hydroxytryptamine. PCA was carried out by the method as described in Inagaki *et al*. (1986). Ten microlitres of 30 fold diluted anti-dinitrofluorobenzene (DNFB) monoclonal IgE antibody was injected into both ears of C57BL/6 mice. After 48 h, PCA was elicited by an intravenous injection of 0.25 mg of dinitrophenylated bovine serum albumin dissolved in 0.5% Evans blue saline solution in a volume of 0.25 ml. Thirty minutes after the challenge, mice were sacrificed by cervical dislocation and both ears were

removed. Both ears were dissolved with 0.7 ml of IN KOH solution at 37°C overnight and 9.3 ml of a mixture of 0.6 NH_3PO_4 solution and acetone (5:13) was added. After vigorous shaking, precipitates were filtered off and the amount of dye was measured colorimetrically at 620 nM. Female C57BL/6 mice received 20 μl of substance P or 5-hydroxytryptamine at a concentration of 10 nM into one ear. As a vehicle control, 20 μl of saline was injected into the other ear. Immediately after the injection of substance P, 5-hydroxytryptamine or saline, 0.25 ml of 0.5% Evans blue in saline was injected intravenously. Extraction and measurement of extravasated dye was carried out as described above for the PCA.

Measurement of immunoglobulins in serum

In order to understand the effect of IP receptor deficiency on immunoglobulin production, mice were immunized by intraperitoneal injection and given repeated inhalations of antigen as described previously in the airway inflammation. Blood was collected at the time before antigen-provocation, and each serum was obtained by centrifugation and stored at −80°C. Antigen-specific IgE, total IgE and total IgG levels in the mouse serum was measured using the ELISA method as described by us (Nagai *et al*., 1999).

In brief, to measure the specific IgE, flat-bottom 96-well microliter plates (Nunc Immuno-Plate I 96-F, Roskilde, Denmark) coated with anti-mouse IgE (Serotec, Oxford, U.K.) were used. One hundred microlitres of diluted sample sera were added to each well, followed by incubation at room temperature for 1 h. After washing with phosphate buffered saline containing 0.1% Tween 20 (T-PBS), 100 μl of diluted biotinylated-OA was added to each well and incubated at room temperature for 1 h. Then, after extensive washing with T-PBS, 100 mg of diluted peroxidase-conjugated streptavidin (Dakopatts a/s, Glostrup, Denmark) was added to each well. The enzymatic reaction was stopped by adding 100 μl of 2 M H_2SO_4 after incubation at room temperature for exactly 30 min. The optical densities of the enzymatic reactions were read using an automatic ELISA plate reader (Titertek Multiscan MCC/340; Lab Systems Oy, Helsinki, Finland) at 492 nm (reference 690 nm). To make a standard curve, sequentially diluted anti-OA IgE monoclonal antibody (mAb; donated by Dr M. Kiniwa, Taiho Pharmaceutical Co. Ltd., Saitama, Japan) was used.

To measure the total IgE and IgG, similar procedures were carried out by using peroxidase-labelled polyclonal anti-mouse IgE (Nordic Immunological Laboratories, Tilburg, The Netherlands) and goat anti-mouse IgG (Capple, Cooper Biomedical Inc., Malvern, PA, U.S.A.).

Cell preparation and cytokine production

To analyse the effect of IP receptor gene deficiency on T-cell function, cytokine production by T cells was examined. Spleen cells and splenic $CD4^+$ T-lymphocytes were used for either antigen-stimulated or anti-CD3 stimulated cytokine production, respectively. The spleen was removed from IP receptor deficient and wild-type mice 12 days after the first immunization, and a dissociated cell suspension (sensitized spleen cells) was prepared and washed twice in RPMI 1640 medium with 5% foetal bovine serum (FBS, Intergen, NY, U.S.A.). The cells (1×10^6) were then suspended in 200 μl of RPMI 1640 medium supplemented with 10% FBS, 10 mM glutamine, 100 u ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 0.05 mM 2-mercaptoethanol, and were cultured in duplicate with or without OA (100 mg ml^{-1}) in a 96-well round-bottom plate at 37°C for 48 h in humidified 5% CO_2/95% air. After the culture, the culture supernatant was collected and centrifuged at $400\times g$ at 4°C. The cell-free supernatants were stored at −80°C until the cytokine assay.

In a separate experiment, the spleen was removed from non-sensitized IP receptor deficient and wild-type mice, and a single-cell suspension was prepared and a T-lymphocyte rich fraction was obtained by centrifugation at $1000\times g$ for 20 min at room temperature using Lympholyte-M (Cedarlane, Ontario, Canada), which was washed twice in PBS with 2 mM EDTA-2Na and 0.5% BSA. The T-lymphocyte rich fraction was treated with magnetic beads conjugated with anti-CD4 (L3T4) monoclonal antibody (Miltenyi Biotec, Bergisch Gladbach, Germany), and purified using a Vario-MACS (Miltenyi Biotec) assembly fitted with a VS^+ column (Miltenyi Biotec). The purified $CD4^+$ T-lymphocytes were washed and counted. The cells (5×10^5) were then resuspended in 1 ml of RPMI 1640 medium as described above, and cultured in triplicate with anti-CD28 mAb (10 μg ml^{-1}, 37.51, Pharmingen, CA, U.S.A.) in the 48-well plate precoated with anti-CD3 mAb (10 μg ml^{-1}, 145-2C11, Pharmingen) at 37°C for 72 h. The cells were washed, counted, and then were cultured in RPMI 1640 medium supplemented with murine IL-2 (50 u ml^{-1}, Genzyme/Tecne, CA, U.S.A.) for 3 days. The cells were then washed, counted, and resuspended in 1 ml of RPMI 1640 medium without IL-2, and re-stimulated with anti-CD3 mAb (10 μg ml^{-1}) for 24 h. The culture supernatant was collected and centrifuged at $400\times g$ at 4°C. The cell-free supernatants were stored at −80°C until the cytokine assay.

Statistical analysis

Values are represented as the mean ± s.e.mean. Statistical significance between two groups was estimated using the two-tailed Student's *t*-test or the Mann–Whitney *U*-test.

Results

Airway inflammation in IP receptor deficient mice

In a previous study, we reported the time course of antigen-induced airway inflammation (Nagai *et al*., 1996). The number of leukocytes and the amount of cytokines in BALF increased significantly after the three inhalations of antigen. They reached a maximum level 8 h after the third antigen inhalation in sensitized mice. In this study, therefore, the BALF study was carried out 8 h after the last antigen inhalation. The number of total cells, eosinophils, neutrophils and lymphocytes in BALF were significantly increased in antigen-inhaled sensitized wild-type mice (Figure 1). Similar increases were observed in antigen challenged sensitized IP receptor deficient mice. The magnitude of increase in the number of leukocytes in IP receptor deficient mice was three or five times more than those in the wild-type mice ($P<0.01$). In the saline treated group, no

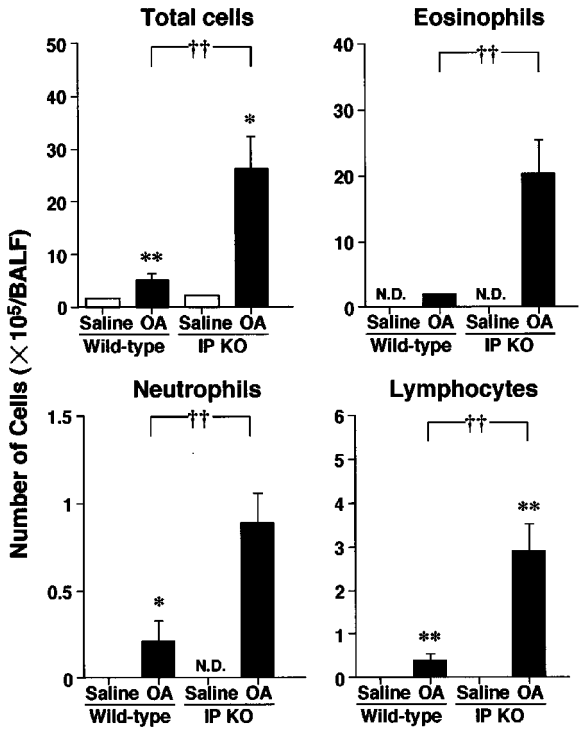

Figure 1 Antigen-induced leukocyte accumulation in bronchoalveolar lavage fluid (BALF) 8 h after the last antigen challenge in 129 × C57BL/6 (F5; wild-type) or IP receptor deficient (KO) mice. Results are presented as the means ± s.e.mean of 4–8 animals. N.D.: not detected, OA: ovalbumin. *, **: $P<0.05$ and 0.01 (*vs* saline; Mann–Whitney *U*-test). ††: $P<0.01$ (*vs* wild-type; Mann–Whitney *U*-test).

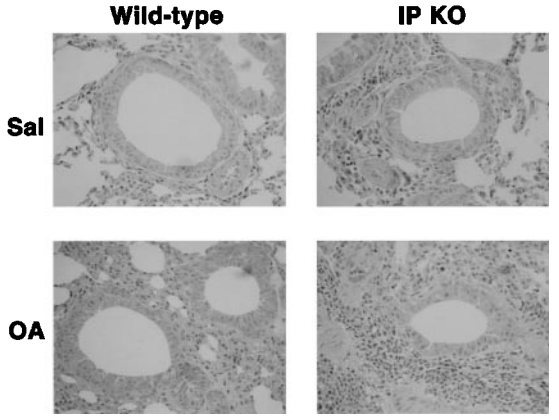

Figure 2 Morphological features of antigen-induced airway inflammation obtained from wild-type or IP receptor deficient (KO) mice. The tissue was stained with H & E (× 100). Representative histology showing profound infiltration of inflammatory cells around small bronchioles of OA challenged IP deficient mice. (A) Wild-type, saline treated, (B) Wild-type, OA treated, (C) KO, saline treated, (D) KO, OA treated.

significant change was observed in both wild-type and IP receptor deficient mice.

In addition to the BALF study, histological studies on lung tissue were carried out. Marked infiltration of eosinophils and mononuclear cells were observed in the peribronchial and perivascular regions of the lung in both wild-type and IP receptor deficient mice (Figure 2). The degree of leukocyte infiltration in the area of inflammatory lesion around pulmonary arterioles and bronchioles was measured under microscopic examination. The number of leukocytes in five different areas of each group was counted. The magnitude of eosinophilic inflammation was more severe in IP receptor deficient mice in comparison with those of wild-type mice.

Simultaneously, the levels of albumin, IL-4 and IL-5 in BALF were measured. The leakage of albumin into the BALF (representing increased vascular permeability) was increased 8 h after the antigen challenge in both groups of animals (Figure 3). The level of albumin in the BALF from IP receptor deficient mice was almost 3.2 fold more than that from wild-type mice, after antigen inhalation ($P<0.05$).

The levels of IL-4 and IL-5 in BALF were significantly increased in both wild-type and IP receptor deficient mice after the antigen challenge compared with those after saline challenge. However, after challenge with antigen, levels of IL-4 and IL-5 in IP receptor deficient mice were increased almost five times and seven times, respectively, higher than those in wild-type mice ($P<0.05$) (Figure 3).

Vascular leakage in the skin

In the skin of wild-type mice, the PCA reaction and substance P (10 nM per site) showed a tendency to increase the dye leakage (Figure 4), whereas 5-hydroxytryptamine (10 nM per site) caused a significant dye leakage in these mice. The amount of extravasated dye in IP receptor deficient mice caused by PCA ($P<0.05$), substance P ($P<0.01$) or 5-hydroxytryptamine ($P<0.05$) was significantly greater than that in wild-type mice treated equivalently.

Antigen-induced increases in serum immunoglobulin levels

The levels of serum OA-specific IgE, total IgE and total IgG were significantly elevated in both wild-type and IP receptor deficient mice after each immunization and antigen inhalation. Figure 5 shows the immunoglobulin levels in the serum obtained 9 days after the secondary sensitization by intraperitoneal injection of OA. There was no significant change in all immunoglobulin levels after the treatment with saline. The level of each immunoglobulin in IP receptor deficient mice was significantly higher than that of wild-type mice ($P<0.05$ or $P<0.01$). OA-specific serum IgE levels in saline-treated mice were below the detection limit (<10 ng ml^{-1}).

To elucidate the mechanism for an increased production of immunoglobulin, the activity of T cells in sensitized and non-sensitized mice was studied by the antigen- or anti-CD3 antibody-induced production of cytokines by spleen cells. The production of either IL-4 or interferon (IFN)-γ was increased after antigen challenge in sensitized wild-type mice as shown in Figure 6A. Production of both cytokines was higher in IP receptor deficient mice after the antigen-stimulation. The amount of IL-4 was almost 3 fold increased with statistical significance ($P<0.01$). IFN-γ was also increased about 3 fold, but without statistical significance. The production of both cytokines by anti-CD3 antibody by splenocytes from non-sensitized mice is shown in Figure 6B. The mean amount of IL-4 increased 2 fold in IP receptor deficient mice, but

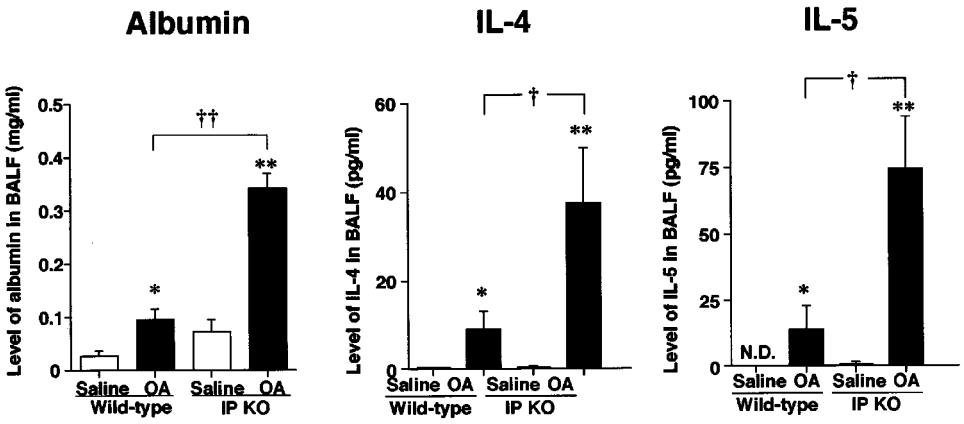

Figure 3 The levels of albumin, IL-4 and IL-5 in bronchoalveolar lavage fluid (BALF) obtained 8 h after the last antigen challenge in wild-type or IP receptor deficient (KO) mice. Results are presented as the mean ± s.e.mean of 4–8 animals. Albumin and cytokine levels were determined using ELISA. N.D.: not detected (< 5 pg ml^{-1}), OA: ovalbumin. *, **: $P<0.05$ and 0.01 (*vs* saline; Mann–Whitney *U*-test). †, ††: $p<0.05$ and 0.01 (*vs* wild-type; Student's *t*-test or Mann–Whitney *U*-test).

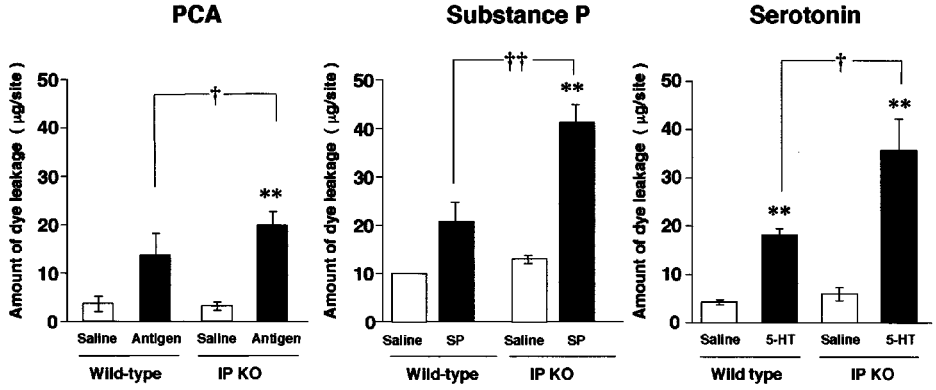

Figure 4 Dye leakage caused by PCA, substance P (10 nM per site) and 5-hydroxytryptamine (5HT; 10 nM per site) in wild-type mice and IP receptor deficient (KO) mice. Each value and bar represents the mean ± s.e.mean of 4–6 mice. **$P<0.01$ (*vs* saline treated group; Student's *t*-test), †, ††: $P<0.05$ and 0.01 (*vs* wild-type; Mann–Whitney *U*-test).

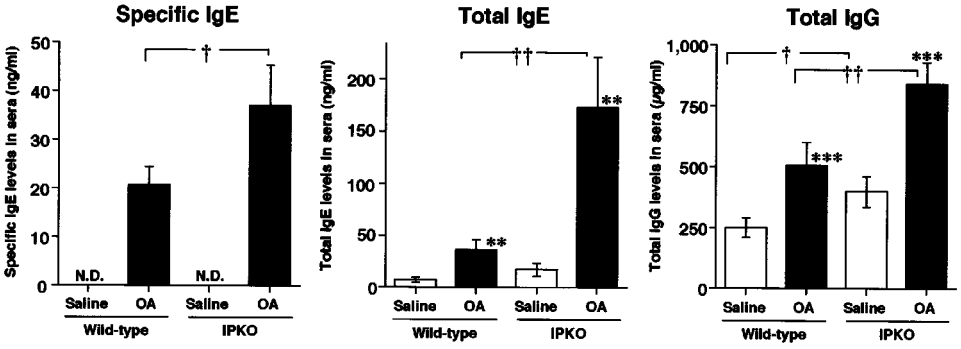

Figure 5 Antigen-induced increases in serum immunoglobulin levels in wild-type and IP receptor deficient (KO) mice. Sera were obtained 9 days after the secondary sensitization with OA. Results are presented as the mean ± s.e.mean of 4–6 animals. Immunoglobulin levels were determined using ELISA. N.D.: not detected (< 1 ng ml^{-1}), OA: ovalbumin. *, **, ***: $P<0.05$, 0.01 and 0.001 (*vs* saline; Student's *t*-test or Mann–Whitney *U*-test). †, ††: $P<0.05$ and 0.01 (*vs* wild type; Student's *t*-test or Mann–Whitney *U*-test).

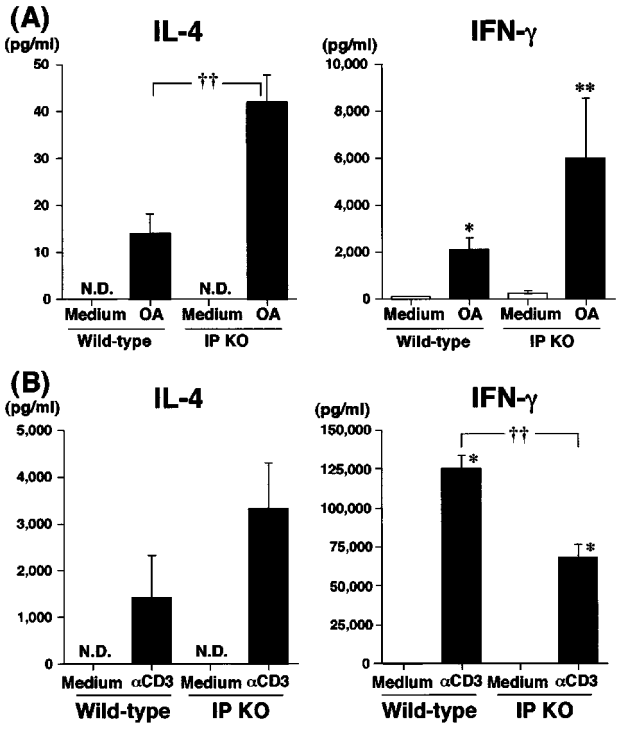

Figure 6 Antigen-induced cytokine production by splenocytes obtained from sensitized IP receptor deficient (KO) mice or wild-type mice (A) and anti-CD3 antibody induced cytokine production by splenic $CD4^+$ T cells from IP receptor deficient or wild-type mice (B). Each level represents the mean ± s.e.mean of 3–5 mice. The sensitized spleen cells were incubated with antigen for 48 h and $CD4^+$ non-sensitized T cells were incubated with anti-CD3 antibody for 24 h. Cytokine levels were measured by ELISA. N.D.: not detected (<5 pg ml^{-1}), N.S.: not significantly different, OA: ovalbumin. *, **: $P<0.05$ and 0.01 (*vs* medium; Student's *t*-test or Mann–Whitney *U*-test). ††: $P<0.01$ (*vs* wild-type; Student *t*-test or Mann–Whitney *U*-test).

without statistical significance. In contrast, IFN-γ production was significantly lower by almost one half, in IP receptor deficient mice ($P<0.01$).

Discussion

In this study, we demonstrated an enhancement of allergic inflammation in the airway and skin of IP receptor deficient mice. In addition, IgE and IgG production were augmented in IP receptor deficient mice. These findings suggest that PGI_2 plays an important suppressive role in allergic inflammation. In previous studies, Murata *et al.* (1997) reported a lowered response in carrageenin-induced inflammation in IP receptor deficient mice, implying a pathological role for PGI_2 in non-allergic inflammation. However, the allergic responses measured in the present study were elevated in IP receptor deficient mice. These differences were caused by several different factors in allergic and non-allergic inflammatory responses, especially the participation of immunological processes.

Regarding the allergic airway inflammation, deficiency of cyclo-oxygenase resulted in the enhancement of airway eosinophilia and bronchial hyper-responsiveness (Gavett *et al.*, 1999), suggesting that cyclo-oxygenase products play a role as a protective factor in allergic airway inflammation. We have shown diminished allergic asthmatic responses in mice, deficient in the DP receptor gene (Matsuoka *et al.*, 2000). Therefore, we can conclude that PGD_2 plays a role as a causative substance in allergic inflammation of airway. In addition to PGD_2, other prostanoids, such as $PGF_{2\alpha}$ and thromboxane A_2, may play pathophysiological roles in allergic bronchoconstriction (Godard *et al.*, 1981; Lahti *et al.*, 1983; Montuschi *et al.*, 1999; Schulman *et al.*, 1981; Yamasaki *et al.*, 1997). In contrast, PGE_2 and PGI_2 were reported to be suppressive mediators in allergic reactions. Therefore, the balance of the production of each PG during the allergic response is important in determining the severity of allergic airway inflammation.

Regarding the role of PGI_2 in cutaneous vascular permeability, contrasting results have been reported. PGI_2 and a PGI_2 analogue inhibited the increase in vascular permeability caused by histamine, 5-hydroxytryptamine, leukotriene B_4 and tumor necrosis factor (TNF)-α (Erlansson *et al.*, 1989; Muller *et al.*, 1987; Suzuki *et al.*, 2001). In contrast, PGI_2 participated in the increase of vascular permeability caused by bacterial lipopolysacharide, bradykinin and carrageenan (Moller & Grande, 1997; Okiji *et al.*, 1989). Moreover, as described previously, Murata *et al.* (1997) reported the suppression of oedema caused by carrageenan in IP receptor deficient mice. These results suggest that the role of PGI_2 in vascular permeability depends on the stimulus inducing the change in permeability. In addition, Jahr & Grande (1996) and Neppl *et al.* (1991) reported the action of PGI_2 on capillary permeability was affected by the concentration of PGI_2. As shown in the present results, IP receptor deficient mice showed an augmentation of vascular permeability caused by antigen challenge, substance P and 5- hydroxytryptamine. Considering together the previous and present findings, we would conclude that PGI_2 plays a protective role in vascular permeability, at least in allergic conditions.

Concerning the effect of PGs on immune responses, little attention has been paid to the role of PGI_2. The present studies employing IP receptor deficient mice indicate enhancement of immunoglobulin production and alteration of T-cell function after allergic reaction. The enhancement of T-cell function is demonstrated in antigen- or anti-CD3 antibody induced cytokine production by spleen cells from IP receptor deficient mice. The antigen-induced production of IL-4 by spleen cells from sensitized IP receptor deficient mice is significantly higher than that from wild-type mice. Moreover, IP receptor deficient mice showed a tendency to increase the production of IFN-γ compared with that from wild-type mice. These results were supported by the *in vivo* experiments, measuring antigen-induced cytokine production in BALF, and showing that amounts of IL-4 and IL-5 in IP receptor deficient mice were greater than that in wild-type mice. In contrast, the anti-CD3 antibody-induced cytokine production by splenocytes from non-sensitized mice resulted in a different pattern. Whereas the production of IL-4 was increased slightly, IFN-γ production was significantly decreased by the disruption of the IP receptor gene. This suggests that the functional activity of Th1 cells in IP receptor deficient mice was congenitally lower than wild-type mice. However, when the antigen was added to sensitized

spleen cells, the Th2 cells are activated significantly. These data suggest the dominance of Th2 response in IP receptor deficient mice over the Th1 response. Since there are many findings to indicate the importance of the Th1 and Th2 balance in allergic reactions (Calder, 2000; Godard *et al*., 1981; Prahalad, 2000; Ray & Cohn, 2000), further experiments are necessary to clarify the role of PGI_2 especially concerning the balance between Th1 and Th2 cells during immunological processes. In addition, the activities of other immunological cells including antigen presenting cells, B cells and others were not studied. The present data only indicate the alteration of T-cell activities in IP receptor deficient mice. Further experiments on the activities of immune competent cells, apart from T, cells in IP receptor deficient mice are necessary.

This study was conducted to investigate the role of PGI_2 in allergic responses by generating these responses in IP receptor deficient mice. Since Murata *et al*. (1997) demonstrated an abrogation of the pharmacological actions of a PGI_2 analogue in IP receptor deficient mice, it is possible to evaluate the role of PGI_2 by using IP receptor deficient mice. However, there are some reports that high concentrations of PGI_2 can activate other prostanoid receptor subtypes (Coleman *et al*., 1989; Dong *et al*., 1986). Further studies employing specific IP receptor agonists will be necessary to confirm the roles of PGI_2 in allergic inflammation.

In summary, the findings of this study suggest an increased allergic response in the airways and skin of IP receptor deficient mice. The enhancement of this allergic response is closely related to an increase in capillary permeability in each tissue. Moreover, IP receptor deficient mice showed a higher immune response to antigen, in terms of the production of serum IgE and IgG and T cell reactivity, especially the Th2 response. These findings suggest a protective role for PGI_2 in the allergic response.

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(Received February 27, 2002
Revised June 26, 2002
Accepted July 9, 2002)