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Opposing Immunomodulatory Roles of Prostaglandin D\(_2\) during the Progression of Skin Inflammation

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The effects of PGD\(_2\) are extremely context dependent. It can have pro- or anti-inflammatory effects in clinically important pathological conditions. A greater mechanistic insight into the determinants of PGD\(_2\) activity during inflammation is thus required. In this study, we investigated the role of PGD\(_2\) in croton oil–induced dermatitis using transgenic (TG) mice overexpressing hematopoietic PGD synthase. Administration of croton oil caused tissue swelling and vascular leakage in the mouse ear. Compared with wild-type animals, TG mice produced more PGD\(_2\) and showed decreased inflammation in the early phase, but more severe manifestations during the late phase. Data obtained from bone marrow transplantation between wild-type and TG mice indicated that PGD\(_2\) produced by tissue resident cells in the TG mice attenuated early-phase inflammation, whereas PGD\(_2\) produced from hematopoietic lineage cells exacerbated late-phase inflammation. There are two distinct PGD\(_2\) receptors: D-prostanoid receptor (DP) and chemoattractant receptor–homologous molecule expressed on Th2 cells (CRTH2). In TG mice, treatment with a DP antagonist exacerbated inflammation in the early phase, whereas treatment with a CRTH2 antagonist attenuated inflammation (DP) and chemoattractant receptor–homologous molecule expressed on Th2 cells (CRTH2). In vitro experiments showed that DP agonism enhanced vascular endothelial barrier formation, whereas CRTH2 agonism stimulated neutrophil migration. Collectively, these results show that when hematopoietic PGD synthase is overexpressed, tissue resident cell–derived PGD\(_2\) suppresses skin inflammation via DP in the early phase, but hematopoietic lineage cell–derived PGD\(_2\) stimulates CRTH2 and promotes inflammation during the late phase. In vitro experiments showed that DP agonism enhanced vascular endothelial barrier formation, whereas CRTH2 agonism stimulated neutrophil migration. Collectively, these results show that when hematopoietic PGD synthase is overexpressed, tissue resident cell–derived PGD\(_2\) suppresses skin inflammation via DP in the early phase, but hematopoietic lineage cell–derived PGD\(_2\) stimulates CRTH2 and promotes inflammation during the late phase. DP-mediated vascular barrier enhancement or CRTH2-mediated neutrophil activation may be responsible for these effects. Thus, PGD\(_2\) represents opposite roles in inflammation, depending on the disease phase in vivo. *The Journal of Immunology, 2014, 192: 459–465.

Prostaglandins are metabolites of arachidonic acid generated by cyclooxygenase (COX) that are synthesized and released upon encountering injurious stimuli. COX-mediated PG synthesis is generally categorized as a proinflammatory event. For instance, a major PG, PGE\(_2\), triggers common inflammatory symptoms, including swelling, pain, and fever (1, 2). Another prostaglandin, TXA\(_2\), promotes platelet aggregation and vascular contraction (3). Clinically, inhibition of COX-mediated PG synthesis has been applied to treat several types of inflammatory diseases, including arthritis (4) and colorectal cancer (5).

PGD\(_2\) is reported to promote sleep and to mediate inflammatory responses. PGD\(_2\) synthesis is of two types: lipocalin-type PG synthase and hematopoietic PGD synthase (H-PGDS). Lipocalin-type PGD synthase is expressed mainly in the arachnoid and choroid membrane, and is implicated in sleep (6) and pain promotion (7). In contrast, H-PGDS is expressed mainly in immune cells, such as mast cells and Th2 lymphocytes, and contributes to various inflammatory responses in peripheral tissues (8). PGD\(_2\) exerts its effects via two types of receptors: D-prostanoid receptor (DP) and chemoattractant receptor–homologous molecule expressed on Th2 cells (CRTH2). The half-life of PGD\(_2\) in blood is short (0.9 min) (9). Several in vitro and in vivo studies have shown that PGD\(_2\) is quickly degraded to several products through enzymatic and/or nonenzymatic pathways (10, 11), and that these products also stimulate PGD\(_2\)-dependent pathways. Whereas PGD\(_2\) and its degradation product, PGJ\(_2\), can bind DP and CRTH2 equally, many of the other products, such as 9α,11β-PGF\(_2\), 13,14-dihydro-15-keto-PGD\(_2\) (DK-PGD\(_2\)), and 15-deoxy-Δ\(_{12,14}\)-PGD\(_2\) (15d-PGD\(_2\)), possess higher binding affinity for CRTH2 (12–14). J-ring PGs also exhibit bioactivity, as they are ligands for the peroxisome proliferator–activated receptor-γ (15–17). Although the pathophysiological implications of each PGD\(_2\) metabolite in vivo remain elusive, various activities have been ascribed to these degradation products.

Reports have proved contradictory regarding whether PGD\(_2\) is pro- or anti-inflammatory. For instance, administration of PGD\(_2\) or a DP agonist has anti-inflammatory effects in rat bowel inflammation (18)
and mouse atopic dermatitis (19). DP receptor agonism inhibited eosinophil migration (20) and dendritic cell activation (21) in a mouse model of asthma. Our group also proved that PGD₂ suppressed mouse acute lung inflammation and tumor growth by inhibiting vascular permeability and subsequent angiogenesis via DP stimulation (22, 23). According to these observations, the PGD₂-DP axis is likely to exert anti-inflammatory effects. In contrast, several other groups showed that PGD₂ signaling mediates proinflammatory responses. For example, administration of an H-PGDS inhibitor improved mouse airway inflammation (24). Furthermore, treatment with a CRTH2 agonist aggravated atopic dermatitis and asthma in mice, whereas intratracheal administration of PGD₂ promoted eosinophil migration in rats (25, 26). Thus, PGD₂ is likely to regulate inflammation differently, depending on the disease type.

The overall outcome of an inflammatory response is determined by multiple factors, including the type of mediator-producing cell/effector cell, the precise effector cell function, the amount of inflammatory signal produced, and the identity of the receptor receiving the stimulus. Furthermore, these factors vary according to the site and phase of inflammation. Because PGD₂ and its metabolites exert various actions through several signal pathways, detailed studies investigating where and how PGD₂ is produced, and its effects in discrete phases of discrete diseases, are indispensable for the design of future therapies.

In the current study, we compared inflammatory reactions in the skin between transgenic (TG) mice overexpressing H-PGDS and wild-type (WT) mice to evaluate the contribution of PGD₂ at each stage of disease progression. We demonstrate that PGD₂-DP signaling originating in tissue resident cells alleviates vascular permeability in the early phase of croton oil–induced dermatitis, and that PGD₂-CRTH2 signaling in infiltrating hematopoietic cells promotes inflammation during the late phase of dermatitis.

Materials and Methods

Reagents
Croton oil, vascular endothelial growth factor (VEGF), and Evans blue were from Sigma-Aldrich; Triton X-100, paraformaldehyde, formamide, and methyl acetate were from Wako Pure Chemical; PGD₂, BW 245C, BW A868C, CAY10471, AL 8810, SQ29548, 15-deoxy-Δ12,14-PGD₂, 9α,11β-PGF₂, DK-PGD₂, anti–H-PGDS Ab, and anti–COX-2 Ab were from Cayman Chemical; anti–CD45 Ab was from Millipore; and penicillin-streptomycin was from Life Technologies.

Mouse dermatitis model
All animal experiments were performed in accordance with the guidelines of the University of Tokyo. TG mice with FVB background were generated and bred as described previously (30). Briefly, excised ears were quickly frozen into liquid nitrogen and homogenized in ethanol containing 0.02% HCl, and the samples were separated by HPLC. An API3200 triple-quadruple tandem mass spectrometer (Applied Biosystems) was used.

Immunostaining
Ear tissues were fixed in 4% paraformaldehyde for 2 d and embedded in paraffin. Sections of 4-μm thickness were stained with H&E. For immunostaining, 5-μm-thick frozen sections were used. After permeabilization/blocking with 0.1% Triton X-100 and 5% normal goat serum for 30 min, sections were labeled with anti–H-PGDS Ab (1:400), anti–COX-2 Ab (1:250), or anti-CD45 Ab (1:400) overnight at 4°C. Then, the sections were labeled with secondary Ab and DAPI. The images were captured using an Eclipse E800 fluorescence microscope (Nikon), and the number of CD45⁺ cells was counted in three randomly chosen fields in each slide.

Measurement of transendothelial electric resistance
Bovine thoracic aortas were purchased from a slaughterhouse. Bovine aortic endothelial cells (BAECs) were isolated and cultured in DMEM containing 10% FBS. BAECs (2.5 × 10⁶) were seeded into each well of the micro-electronic sensor, and then transendothelial electric resistance (TER) was measured by xCELLigence real time cell analyzer DP system (Roche). Confluent monolayers of BAECs were serum starved for 10 h before the experiment. TER was measured every 30 s and then normalized to the initial value.

Isolation of neutrophil and chemotaxis assay
Narrow cavities of the tibias and femurs of 8-wk-old mice were flushed with DMEM. Neutrophils were isolated by centrifugation over discontinuous Percoll gradients. For the chemotaxis assay, a modified Boyden chamber for 24 h. Dye content was measured by reading at 610 nm in a spectrophotometer (Wallac 1420, PerkinElmer) and normalized to ear dry weight.

Measurement of PGs
Contents of PGD₂, PGE₂, and TXA₂ in ears were measured as previously described (30). Briefly, excised ears were quickly frozen into liquid nitrogen and homogenized in ethanol containing 0.02% HCl, and the samples were separated by HPLC. An API3200 triple-quadruple tandem mass spectrometer (Applied Biosystems) was used.

FIGURE 1. Croton oil–induced skin inflammation in H-PGDS–overexpressing mice. Croton oil (2.5%, 2 h or 6 h) or VEGF (30 ng, 35 min) was administered to mouse ears. Evans blue dye (30 mg/kg) was injected and circulated for 30 min. (A) Representative photographs show croton oil–induced inflammation. Skin thickness (B) or dye extravasation (C) of the inflamed ears was quantified (n = 5–8 each, *p < 0.05 compared with WT). The concentration of PGD₂ (D) or PGE₂ (E) in inflamed ears was determined by a mass spectrometer (n = 5, *p < 0.05 compared with WT).
with 8-μm pores (BD Falcon) was used. Stimulants were added to the bottom chamber, and inhibitors were added to both the upper and the bottom chambers. Isolated neutrophils (2 × 10^5 cells) were applied to the upper chambers. After 2 h, cells on the membrane were fixed and stained with Giemsa solution. The number of cells from five randomly chosen fields (×200) on the lower side of the membrane was counted.

Data representation and statistical analysis

The results are expressed as mean ± SEM. Statistical evaluations of the data were performed using an unpaired Student t test for comparisons between two groups and by one-way ANOVA followed by a Dunnett test or Tukey test for comparison between more than two groups. A p < 0.05 was taken as significant.

Results

Inflammatory responses in the presence of H-PGDS overexpression

In WT mice, administration of 2.5% croton oil caused ear swelling (Fig. 1B) and increased dye extravasation, an index of vascular permeability (typical pictures are shown in Fig. 1A, and dye leakages are quantified in Fig. 1C). These responses peaked 6 h after the stimulation and settled in 36 h (ear swelling, 6 h, 0.146 ± 0.013 mm; 36 h, 0.012 ± 0.005 mm, n = 5 each).

Pretreatment with a pan-COX inhibitor, indomethacin, inhibited these responses (Supplemental Fig. 1), suggesting the contributions of PG production to the inflammation. TG mice showed reduced severity of ear swelling and less vascular leakage at the early phase (2 h after the stimulation), but showed more severe inflammation than WT mice at the late phase (6 h post treatment). WT and TG mice responded similarly to treatment with VEGF, which directly stimulates vascular leakage without activation of the arachidonate cascade (Fig. 1C, right bars).

Administration of 2.5% croton oil induced PGD2 production in both WT and TG mice (Fig. 1D). PGD2 production in TGs, compared with that in WT mice, was significantly higher during the experiments. The concentration of another prostanoid, PGE2, did not differ between the lines (Fig. 1E).

Infiltrated leukocytes expressed H-PGDS

As shown by the H&E staining (Fig. 2A, upper panels), no morphological difference was observed between WT and TG mice prior to stimulation (0 h). There was a time-dependent ear swelling and infiltration of inflammatory cells upon croton oil administration. Most of the infiltrating cells were neutrophils with segmented nuclei (Fig. 2A, middle and lower panels; see insets). Regarding the ear-swelling data shown in Fig. 1B, TG ears were slightly thinner at the early phase of inflammation, whereas they were much thicker than WT ears at the late phase. Neutrophil infiltration was also accelerated in TG mice at the late phase (Fig. 2A, inset panels).

We next performed immunostaining to define which type of cell expresses H-PGDS in inflamed ears. As shown in Fig. 2B (upper left panels), H-PGDS was not detected in nontreated WT ears. In croton oil–treated WT ears, CD45+ leukocytes expressed H-PGDS (Fig. 2B, middle and lower left panels). TG ears broadly expressed H-PGDS regardless of oil stimulation (Fig. 2B, left panels). These observations are consistent with the fact that human H-PGDS is constitutively expressed in the TG mice from a chicken β-actin promoter. Of interest, oil stimulation increased H-PGDS expression even in TG mice, and in particular in the CD45+ cells. This finding may be due to the elevation of endogenous mouse H-PGDS expression in response to stimulation.

Expression of an inducible type of COX, COX-2, is required to produce PGD2 in the context of inflammation. As shown in Sup-
plemental Fig. 2A and 2B, CD31⁺ vascular endothelial cells constitutively express COX-2 in both lines of mice. Infiltrating CD45⁺ leukocytes (most likely neutrophils) also express COX-2 after stimulation. We thus inferred that these two cell types are the major sources of PGD₂ in this model.

In agreement with the observations from H&E staining, a significant difference was noted in the number of infiltrating leukocytes between the lines during the early phase of inflammation. In contrast, many more leukocytes infiltrated TG ears than WT ears at the late phase (Fig. 2C).

Functional contribution of hematopoietic lineage–derived PGD₂ in dermatitis

We explored the contribution of hematopoietic cell (BM-derived immune cells)– or nonhematopoietic cell (tissue resident cells)–derived PGD₂ in dermatitis using BMT. Irradiated WT recipients with WT BM (WT + WT_BM) exhibited tissue swelling comparable to that in nontreated WT (Supplemental Fig. 3) in response to oil treatment. The BMT procedures (i.e., radiation and BM injection) did not affect the inflammatory responses. At the early phase of inflammation (2 h), no difference was detected in ear swelling between the lines (Fig. 3A), whereas TG transplanted with WT or TG BM (TG + WT_BM and TG + TG_BM) exhibited blunted vascular leakage compared with WT or TG BM (WT + WT_BM and WT + TG_BM) (Fig. 3B). These results suggest that PGD₂ secreted from the tissue resident cells inhibits the inflammation in TG mice during the early phase of dermatitis.

At the late phase, regardless of recipient genotype, mice transplanted with TG BM (WT + TG_BM, TG + TG_BM) showed more severe swelling (Fig. 3C) and vascular leakage (Fig. 3D) in comparison with the mice with WT BM (WT + WT_BM, TG + WT_BM). This finding indicates that PGD₂ from infiltrating hematopoietic cells (most presumably neutrophils) promotes inflammatory responses in the TG during this phase.

The role of PGD₂-DP signaling in the early phase of inflammation

We attempted to clarify how PGD₂ modulated inflammatory responses during early-phase dermatitis. Administration of an H-PGDS inhibitor, HQL-79 (50 mg/kg, i.p.), significantly enhanced tissue swelling (Fig. 4A) and vascular permeability (Fig. 4B) in both lines of mice. Upon H-PGDS inhibition, the degrees of ear swelling and dye extravasation were similar in WT and TG mice. As with H-PGDS inhibition, DP antagonism by BW A868C (1 mg/kg, i.p.) also promoted inflammatory responses in both WT and TG mice to the same degree (Fig. 4C, 4D).

Thus, the DP-mediated signal most likely contributed to the immunosuppressive reaction of PGD₂ in early-phase inflammation. In support of this idea, DP⁻/⁻ mice were more responsive to croton oil stimulation than were WT mice, whereas CRTH₂⁻/⁻ mice exhibited responses comparable to those of WT mice. Additive treatment with PGD₂ (1 mg/kg, i.p.) inhibited the oil-induced vascular leakage in WT and CRTH₂⁻/⁻ mice, but not DP⁻/⁻ mice (Fig. 4E). Furthermore, administration of a DP agonist, BW 245C (1 mg/kg, i.p.), strongly inhibited vascular leakage in WT ears (Fig. 4F).

The role of PGD₂-CRTH2 signaling in late-phase inflammation

We next assessed the contribution of PGD₂ to late-phase dermatitis. As shown in Fig. 5A and 5B (ear swelling and dye leakage, respectively), inhibition of H-PGDS by HQL-79 (50 mg/kg, i.p.) tended to increase the scores in WT mice, whereas it attenuated them in TG mice. As shown in Fig. 5C and 5D (both data are shown as a ratio to nontreated ears (only with croton oil administration) (n = 4–6, *p < 0.05 and †p < 0.05, compared with nontreated WT ears or CRTH₂⁻/⁻ ears).
as a ratio to nontreated), DP antagonism (BW A868C, 1 mg/kg i.p.) promoted, and CRTH2 antagonism (CAY10471, 1 mg/kg, i.p.) tended to attenuate, the oil-induced inflammation in both lines of mice. The DP-mediated anti-inflammatory reaction was dominant in WT mice, whereas the CRTH2-mediated proinflammatory signal appeared to counteract DP-mediated responses in TG mice.

We could obtain consistent observations using gene-deficient mice. As shown in Fig. 5E, WT and CRTH2−/− mice represented comparable vascular leakage responding to sole oil treatment (6 h). DP deficiency significantly increased vascular leakage even in late-phase dermatitis. Additional treatment with PGD2 increased vascular permeability in WT and increased slightly, but not significantly, in DP−/− mice. In contrast, PGD2 treatment significantly decreased vascular permeability in CRTH2−/− mice.

As described above, PGD2 is rapidly degraded into several products in blood (10, 11). These products possibly accumulate locally and preferentially stimulate CRTH2-mediated proinflammatory signaling according to disease progression. Indeed, treatment with the major PGD2 metabolites DK-PGD2 and 9α,11β-PGF2 (10 ng per ear) significantly enhanced vascular leakage (Fig. 5F).

The effect of PGD2 on vascular permeability and neutrophil migration in vitro

We examined the effect of PGD2 on vascular endothelial permeability by measuring TER in vitro. In line with the in vivo data showing DP-mediated vascular barrier enhancement (Fig. 4E), treatment with PGD2 (10 μM) or BW 245C (1 μM) elevated TER, indicating barrier enhancement (typical responses are shown in Fig. 6A and summarized in Fig. 6B). These effects were completely inhibited by DP antagonism (BW A868C, 10 μM, 30 min before PGD2 administration). A CRTH2 agonist, DK-PGD2 (1 μM), which enhanced vascular permeability in vivo, did not change TER in vitro. In addition, 9α,11β-PGF2 (1 μM) did not affect endothelial barrier formation in vitro (Fig. 6B).

In the transmembrane migration assay, isolated neutrophils migrated toward a solution of 5 nM leukotriene B4 added into the lower chamber (Fig. 6C), as previously reported (31). Stimulation with PGD2 or a DK-PGD2 (1 μM), 9α,11β-PGF2 (100 nM), also induced neutrophil migration; this was abolished by CRTH2 antagonism (CAY10471, 1 μM).

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Discussion
Using genetically modified mice that overexpress H-PGDS, we demonstrated that PGD2 assumes both pro- and anti-inflammatory roles according to the progression of inflammation. Specifically, upon initiation of croton oil–induced dermatitis, PGD2 produced from tissue resident cells exhibits an anti-inflammatory action through DP-mediated signaling. At the progression phase, PGD2, and possibly its degradation products secreted from infiltrating leukocytes, promote inflammation through CRTH2-mediated signaling (Supplemental Fig. 4).

At the early phase of dermatitis, secreted PGD2 had anti-inflammatory activity in both WT and TG mice, which is attributable to DP-mediated signaling. TG showed relatively strong anti-inflammatory reactions. These phenomena are presumably due to the higher level of PGD2 production in TG ears. The experiments using BMT revealed that PGD2 produced from tissue resident cells contributes to the anti-inflammatory reaction during the early phase. Given that vascular endothelial cells constitutively express COX-2 (which is indispensable for PGD2 production), endothelial cell–derived PGD2 is likely to control inflammation in early-phase dermatitis.

We observed that DP-mediated signaling contributes to the anti-inflammatory role of PGD2 by enhancing endothelial barrier formation. We previously reported that vascular endothelial cells express DP and its agonism inhibits vascular leakage in the inflamed lung (22) and growing tumor (23), in agreement with our current observations. The underlying molecular mechanism associated with stimulation of DP is tightening of endothelial cell-to-cell junctions through the cAMP/protein kinase A/Rac signaling pathway (32).

Of interest, in contrast to its anti-inflammatory role in the early phase, secreted PGD2 showed proinflammatory effects in the late phase. When compared with the ears of WT animals, the ears of TG mice had more severe inflammation with increased PGD2 production. BMT and morphological studies suggested that infiltrating leukocytes produced PGD2 and promoted tissue swelling and vascular hyperpermeability. Considering the fact that tissue contents of PGD2 were similar in the early and late phases in each line of mice (Fig. 1D), the site of production seems to be more crucial than the amount of PGD2 for PGD2-mediated immune responses.

We further demonstrated that CRTH2 signaling mediates the proinflammatory effects of PGD2. This activity is likely because many of the PGD2 degradation products potentially bind CRTH2 (12). Further investigation is required to assess the contribution of each product to the pathogenesis of inflammation, especially in vivo. However, it is possible that sustained local inflammation leads to the accumulation of PGD2 metabolites, which further accelerate inflammation by acting as CRTH2 ligands.

Our data show that both a CRTH2 agonist (DK-PGD2) and a major PGD2 metabolite (9α,11β-PGF2) enhanced vascular permeability in vivo, but they did not directly influence endothelial barrier formation in vitro. Previous studies showed that CRTH2 stimulation promotes the migration of BM-derived hematopoietic lineage cells such as TH2 lymphocytes and eosinophils (33). Consistently, CRTH2 agonist stimulated migration of neutrophils in vitro. In addition, infiltration of neutrophils was accelerated in inflamed TG ears. Neutrophils produce a variety of bioactive agents, including cytokines and reactive oxygen, that can stimulate the vascular bed (34). Thus, neutrophil-derived PGD2 may promote neutrophil infiltration/migration through CRTH2 signaling, and as a result, bioactive substances released from infiltrating neutrophils could promote inflammation, at least partially, by disrupting the vascular barrier.

Our data showing that the PGD2–DP signaling axis mediates anti-inflammatory reactions imply that DP agonism may be a strategy for treatment of various inflammatory diseases. Conversely, we also showed in this article that activation of the PGD2–CRTH2 signal axis in inflammatory cells promotes inflammation. These situations probably occur in diseases that are accompanied by local accumulation of PGD2–secreting immune cells, such as allergic inflammation with mast cell and eosinophil accumulation. H-PGDS inhibition or CRTH2 antagonism might be beneficial for these pathological conditions.

Inflammation is an indispensable response for the rejection of foreign substances and for tissue regeneration, and it occurs throughout the body. As key players in this response, PGs are likely produced and received by almost all types of cells. To understand and manage various types of inflammatory diseases, a detailed evaluation of the role of individual PG types with respect to their source and effects in each phase of disease is an absolute requirement. We suggest that our use of the H-PGDS TG mice that overexpress H-PGDS with a β-actin promoter provides significant insight in this regard. The system is not without caveats, however, because the inflammatory symptoms observed in the TG mice were induced owing to exogenous expression of H-PGDS driven by a nonnative promoter. However, this mouse model highlighted the role of PGD2 in inflammation and allowed us to provide deep insights into the paradoxical action of PGD2.

In summary, the Janus-like behavior of PGD2 with regard to its pro- and anti-inflammatory effects in vivo was determined by an exquisite balance of multiple factors, which included the site of its production and the quantity of PGD2 and related degradation products.

Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Figure legends

Supplemental Figure 1. Treatment with a pan-COX inhibitor indomethacin inhibited Croton oil-induced tissue swelling and vascular leakage in WT ears. Indomethacin (10 mg/kg, i.p.) was added 30 min before the stimulation. Skin thickness (A) or dye extravasation (B) of the inflamed ears was assessed (n = 5 each, *, p < 0.05).

Supplemental Figure 2. Vascular endothelial cells and leukocytes express COX-2 protein. Anti-CD31 or anti-CD45 antibody was used to label endothelial cells (A) or leukocytes (B), respectively. Representative pictures of 4 independent experiments are shown. Scale bar: 20 μm.

Supplemental Figure 3. BMT procedures did not affect skin inflammatory responses. Ear thickness was assessed 2 or 6 h after Croton oil treatment (n = 5 each, n.s.; no significant difference).

Supplemental Figure 4. Summary of skin inflammation in the H-PGDS-overexpressing mouse. At the early phase, tissue resident cell-derived PGD₂ suppress skin inflammation through DP-mediated endothelial barrier enhancement. At the late phase, in contrast, hematopoietic lineage cell-derived PGD₂ promotes inflammation through CRTH2-mediated neutrophil accumulation.
Supplemental Figure 1

A

- Ear swelling (mm)
- None
- Indomethacin
- WT
- TG

B

- Evans blue (mg/g)
- None
- Indomethacin
- WT
- TG

* Significant difference
Supplemental Figure 3

[Graph showing ear swelling over time for WT and WT+WTBM groups.]

- Ear swelling (mm)
- WT
- WT+WTBM
- n.s.

Time points:
- 2 h
- 6 h

Comparison between groups indicates significant differences.
Supplemental Figure 4

Early phase

Inflammatory stimuli

PGD$_2$  
Permeability

Leukocyte

Endothelial cell

Late phase

CRTH2  
PGD$_2$

CRTH2  
PGD$_2$

Migration