

Letter to the Editor

Mast cell-derived prostaglandin D₂ attenuates anaphylactic reactions in mice

To the Editor:

Anaphylaxis is a severe and life-threatening allergic reaction. As of the last decade, 0.05% to 2% of the general population worldwide experienced an anaphylactic reaction during their lifetime, and approximately 2% of these subjects died as a result of the reaction.¹

Clinical and experimental studies have shown that vascular hyperpermeability is a hallmark of anaphylaxis.^{2,3} Activated mast cells release vasoactive mediators, such as histamine and platelet-activating factor, which induce the initial anaphylactic response of vascular hyperpermeability by causing vasodilation, endothelial barrier disruption, or both. Systemic vascular hyperpermeability and plasma leakage into local tissues lead to systemic reactions, including increased hematocrit levels and hypotension.

Prostaglandin D₂ (PGD₂) is produced by the activity of COX and hematopoietic PGD synthase (H-PGDS). Because a previous

study demonstrated that mast cells strongly express H-PGDS and produce PGD₂ on a large scale,⁴ we hypothesized that mast cell-derived PGD₂ makes some pathophysiologic contribution to anaphylaxis, although the exact nature of this contribution is unknown.

We first examined anaphylactic vascular hyperpermeability under conditions in which PGD₂ was present or absent. Intravenous injection of the antigen dinitrophenyl (DNP) into the DNP-IgE-sensitized mouse ear induced the extravasation of intravenously injected dye in wild-type (WT) mice (Fig 1, A and B). Intradermal injection of compound 48/80 (C48/80) also induced extravasation in WT mice (Fig 1, C and D). A higher degree of dye extravasation was observed in H-PGDS-deficient (*Hpgds*^{-/-}) mice than in WT mice (Fig 1, A and B). *Hpgds*^{-/-} mice also exhibited a stronger increase in hematocrit levels, more severe hypotension, and deeper and longer hypothermia compared with WT mice in response to systemic mast cell-activating stimulation (Fig 1, E and F, and see Fig E1 in this article's Online Repository at www.jacionline.org). In contrast, overexpression of H-PGDS (*HTG*) attenuated the C48/80-induced

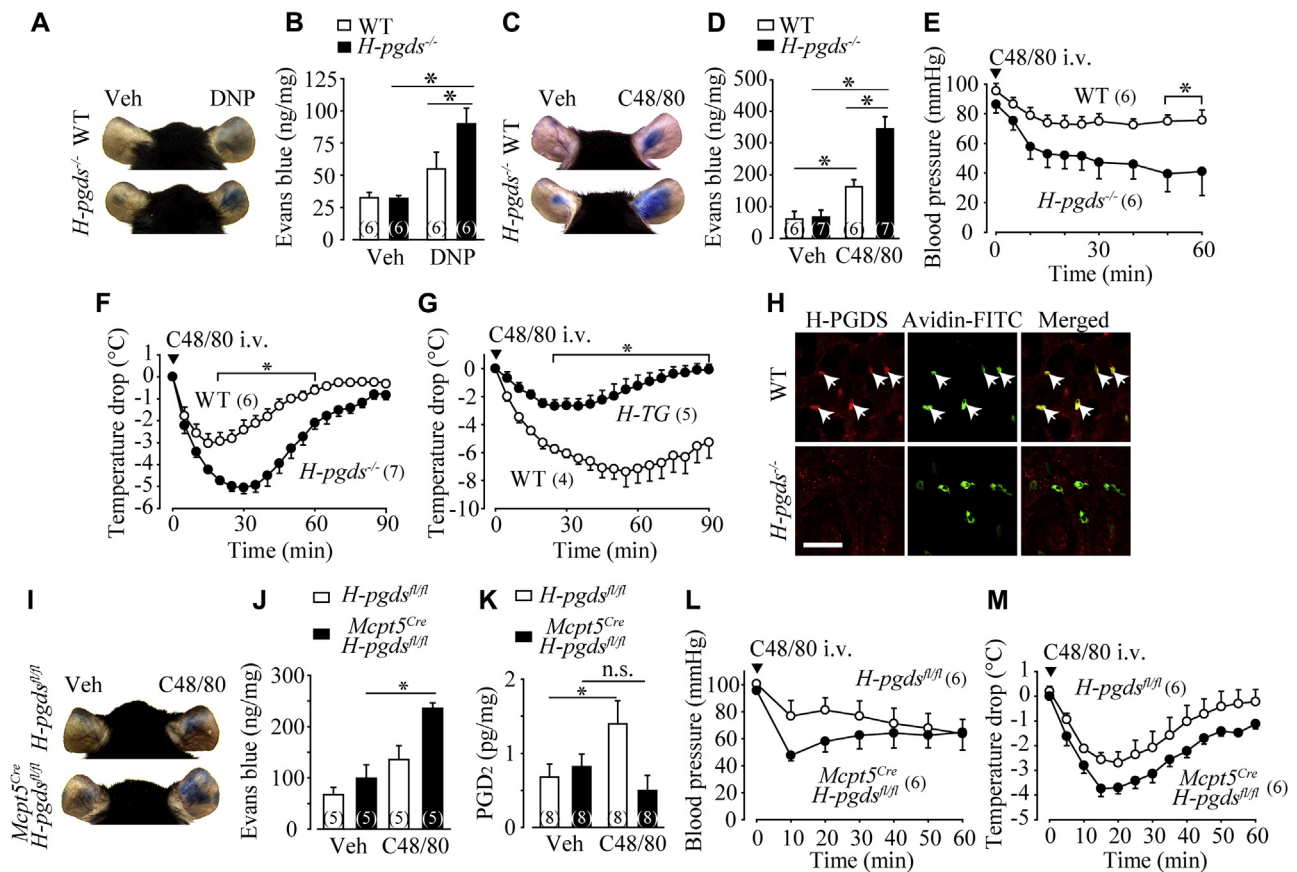


FIG 1. Systemic and mast cell-specific H-PGDS deficiency exacerbates anaphylactic reactions. **A-G,** Antigen/antibody-induced (Fig 1, A and B) and C48/80-induced (Fig 1, C and D) dye extravasation. **E-G,** Hypotension and hypothermia (Fig 1, E) in WT and *Hpgds*^{-/-} (Fig 1, F) and FVB-background WT and *HPGDS*-TG (Fig 1, G) mice. **H,** Whole-mount ear sections of WT and *Hpgds*^{-/-} mice stained with H-PGDS (red) and mast cells (green). Bar = 50 μ m. **I-M,** C48/80-induced (Fig 1, I and J) dye extravasation, PGD₂ production (Fig 1, K), hypotension (Fig 1, L), and hypothermia (Fig 1, M) in *Hpgds*^{fl/fl} and *Mcpt5*^{Cre}*Hpgds*^{fl/fl} mice. Numbers of analyzed animals are shown in parentheses. Data are obtained in 3 independent experiments and are represented as means $\pm$ SEMs. **P* < .05. i.v., Intravenous.

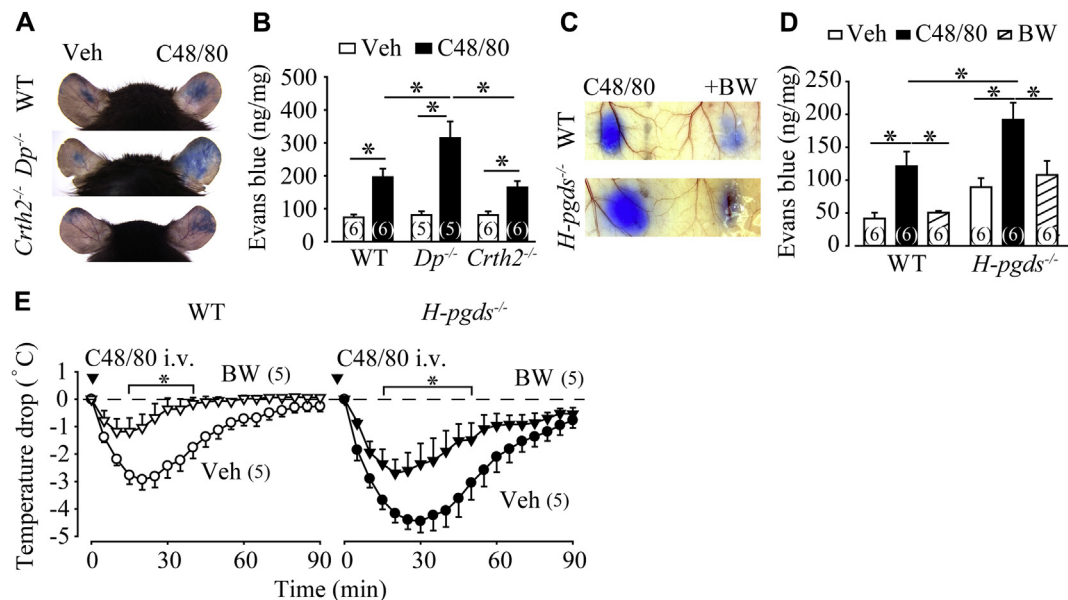


FIG 2. DP receptor mediates the attenuation of anaphylaxis by PGD₂, and its signal enhancement inhibits anaphylaxis. **A** and **B**, C48/80-induced dye extravasation in the mouse ear. **C–E**, Effect of DP agonist (BW245C) on C48/80-induced (Fig 2, **C** and **D**) dye extravasation on back skin and hypothermia (Fig 2, **E**). Numbers of analyzed animals are given in parentheses. Data are obtained in 3 independent experiments and are represented as means $\pm$ SEMs. * $P < .05$. i.v., Intravenous.

hypothermia (Fig 1, **G**). These results suggest that H-PGDS-derived PGD₂ inhibits vascular hyperpermeability and systemic responses in patients with anaphylaxis.

Mast cell-induced anaphylactic responses are largely attributable to histamine release and subsequent nitric oxide (NO) production from endothelial cells.^{5,6} C48/80 stimulation produced comparable increases in plasma levels of histamine in the 2 lines of mice (see Fig E2, **A**, in this article's Online Repository at www.jacionline.org), and pretreatment with the histamine H₁ receptor blocker diphenhydramine abolished the C48/80-induced hyperpermeability and hypothermia (see Fig E2, **B–D**). H-PGDS deficiency did not influence the sensitivities to histamine (see Fig E2, **E–G**). Lack of the endothelial NO synthase gene also abolished the C48/80-induced anaphylactic responses (see Fig E3, **A** and **B**, in this article's Online Repository at www.jacionline.org). Moreover, pretreatment with the NO synthase inhibitor N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) led to a nonsignificant inhibition of dye extravasation and hypothermia in WT mice, whereas it significantly inhibited the responses in *Hpgds*^{-/-} mice (see Fig E3, **C–E**), suggesting increased NO production in the PGD₂-absent condition.

Immunostaining results indicated that mast cells localized with α -smooth muscle actin (α -SMA)-positive and platelet endothelial cell adhesion molecule (PECAM) 1-positive vessels and that their numbers were comparable between WT and *Hpgds*^{-/-} mice (see Fig E4, **A** and **B**, in this article's Online Repository at www.jacionline.org). The perivascular mast cells strongly expressed H-PGDS in the ears of WT but not *Hpgds*^{-/-} mice (Fig 1, **H**). We then generated mast cell-specific H-PGDS-deficient mice (*Mcpt5*^{Cre}*Hpgds*^{fl/fl}) by crossing mast cell protease 5 (MCPT5)-Cre transgenic mice and H-PGDS floxed mice (see Fig E4, **C–E** and Fig E7 in this article's Online Repository at www.jacionline.org). Compared with the control mice (*Hpgds*^{fl/fl}), *Mcpt5*^{Cre}*Hpgds*^{fl/fl} mice exhibited enhanced vascular hyperpermeability

without PGD₂ production in response to C48/80 (Fig 1, **I–K**). On the other hand, *Mcpt5*^{Cre}*Hpgds*^{fl/fl} mice exhibited a slight but nonsignificant increase in C48/80-induced hypotension and hypothermia compared with the *Hpgds*^{fl/fl} mice (Fig 1, **L** and **M**).

There are 2 major types of mast cells: connective tissue-type mast cells (CTMCs) and mucosal-type mast cells (MMCs). CTMCs typically reside in the skin and the peritoneal cavity, whereas MMCs are predominant in the mucosal layer of the lung and intestine. Because the protease MCPT5 is mainly expressed in CTMCs but is rarely expressed in MMCs, *Mcpt5*^{Cre}*Hpgds*^{fl/fl} mice lack H-PGDS in CTMCs. *MCPT5*^{Cre}*iDTR*⁺ mice, in which CTMCs are selectively depleted by diphtheria toxin, exhibit a strong reduction of anaphylactic hypothermia while still exhibiting a small response because of residual MMCs.⁷ MMC-derived PGD₂ can also be involved in anaphylaxis suppression.⁸

We next examined the contribution of 2 distinct PGD₂ receptors to the attenuation of anaphylaxis. Systemic DP deficiency (*Dp*^{-/-}, see Fig E7) but not CRTH2 receptor deficiency (*Crth2*^{-/-}) significantly increased dye extravasation compared with that seen in WT mice (Fig 2, **A** and **B**). Administration of PGD₂ or a DP agonist inhibited the C48/80-induced vascular hyperpermeability (Fig 2, **C** and **D**, and see Fig E5 in this article's Online Repository at www.jacionline.org). There is another endogenous DP ligand, PGD₁, which has much lower affinity than PGD₂. However, the high dose of PGD₁ (1 μ g) did not inhibit anaphylactic hyperpermeability (see Fig E5). The DP agonist also attenuated hypothermia in both mouse lines (Fig 2, **E**). Although it is reported that DP activation induces vasodilation in isolated vessels of the human lung,⁹ it did not influence the blood pressure of mice in this regimen (see Fig E6 in this article's Online Repository at www.jacionline.org). Anaphylactic vascular hyperpermeability depends on endothelial G_q/G₁₁/intracellular calcium ([Ca²⁺]_i)-induced NO production.^{3,6} Activation of the Gs-coupled DP receptor increases intracellular cyclic AMP, which

activates protein kinase A and decreases $[Ca^{2+}]_i$ levels. Results from the experiments using L-NAME indicated that there was increased NO production in *Hpgds*^{-/-} mice, and thus PGD₂-DP signaling can inhibit NO production by decreasing the increased $[Ca^{2+}]_i$ levels in endothelial cells. Additionally, we previously reported that *in vivo* DP activation strongly enhances endothelial barrier formation through the cyclic AMP/protein kinase A/Tiam1/Rac1 pathway.¹⁰ These 2 signaling pathways are possible mechanisms underlying the inhibitory action of PGD₂ on vascular permeability in the setting of anaphylaxis.

Here we clearly demonstrated that mast cell–derived PGD₂ acts as a negative regulator of vascular hyperpermeability and systemic symptoms in a murine model of anaphylaxis *in vivo*. It is notable that mast cells secrete an antianaphylactic mediator and proanaphylactic mediators at the same time; however, the pathophysiologic implication of this characteristic remains unclear. Previous *in vitro* studies showed that mast cell secretion of PGD₂ lasts longer than that of a preformed mediator.⁴ The longer secretion time of PGD₂ might allow PGD₂ to act as a negative regulator to prevent excessive anaphylactic responses.

Tatsuro Nakamura, DVM, PhD^a

Yuki Fujiwara, BSc^a

Ryota Yamada, BSc^a

Wataru Fujii, PhD^b

Taiki Hamabata, BSc^a

Monica Yunkyoung Lee, PhD^c

Shingo Maeda, DVM, PhD^a

Kosuke Aritake, PhD^d

Axel Roers, MD, PhD^e

William C. Sessa, PhD^c

Masataka Nakamura, PhD^f

Yoshihiro Urade, PhD^d

Takahisa Murata, DVM, PhD^a

From ^athe Departments of Animal Radiology and ^bApplied Genetics, Graduate School of Agriculture and Life Sciences, University of Tokyo, Tokyo, Japan; ^cthe Department of Pharmacology, Yale University School of Medicine, New Haven, Conn; ^dthe International Institute for Integrative Sleep Medicine, University of Tsukuba, Ibaraki, Japan; ^ethe Institute for Immunology, Technische Universität Dresden, Dresden, Germany; and ^fthe Human Gene Sciences Center, Tokyo Medical and Dental University, Tokyo, Japan. E-mail: amurata@mail.ecc.u-tokyo.ac.jp.

Supported by a Grant-in-Aid from the Japan Society for the Promotion of Science, the Foundation for Dietary Scientific Research, the Cardiovascular Research Fund, the Kurozumi Medical Foundation, the Naito Foundation, the Suzuken Memorial Foundation, the Takeda Science Foundation, the Nipponham Foundation, the Sapporo Bioscience Foundation, and the Japan Foundation for Pediatric Research.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Tejedor-Alonso MA, Moro-Moro M, Mugica-Garcia MV. Epidemiology of anaphylaxis: contributions from the last 10 years. *J Invest Allergol Clin Immunol* 2015;25:163-75.
2. Fisher MM. Clinical observations on the pathophysiology and treatment of anaphylactic cardiovascular collapse. *Anaesth Intensive Care* 1986;14:17-21.
3. Korhonen H, Fisslthaler B, Moers A, Wirth A, Habermehl D, Wieland T, et al. Anaphylactic shock depends on endothelial Gq/G11. *J Exp Med* 2009;206:411-20.
4. Murakami M, Matsumoto R, Urade Y, Austen KF, Arm JP. c-kit ligand mediates increased expression of cytosolic phospholipase A2, prostaglandin endoperoxide synthase-1, and hematopoietic prostaglandin D2 synthase and increased IgE-dependent prostaglandin D2 generation in immature mouse mast cells. *J Biol Chem* 1995;270:3239-46.
5. Makabe-Kobayashi Y, Hori Y, Adachi T, Ishigaki-Suzuki S, Kikuchi Y, Kagaya Y, et al. The control effect of histamine on body temperature and respiratory function in IgE-dependent systemic anaphylaxis. *J Allergy Clin Immunol* 2002;110:298-303.
6. Cauwels A, Janssen B, Buys E, Sips P, Brouckaert P. Anaphylactic shock depends on PI3K and eNOS-derived NO. *J Clin Invest* 2006;116:2244-51.
7. Scholten J. Novel tools for mast cell research: mast cell-specific Cre-mediated gene inactivation and inducible ablation of mast cells *in vivo* [dissertation]. Köln (Germany): Universität zu Köln; 2009.
8. Lundstrom SL, Saluja R, Adner M, Haeggstrom JZ, Nilsson G, Wheelock CE. Lipid mediator metabolic profiling demonstrates differences in eicosanoid patterns in two phenotypically distinct mast cell populations. *J Lipid Res* 2013;54:116-26.
9. Walch L, Labat C, Gascard JP, de Montpreville V, Brink C, Norel X. Prostanoid receptors involved in the relaxation of human pulmonary vessels. *Br J Pharmacol* 1999;126:859-66.
10. Kobayashi K, Tsubosaka Y, Hori M, Narumiya S, Ozaki H, Murata T. Prostaglandin D2-DP signaling promotes endothelial barrier function via the cAMP/PKA/Tiam1/Rac1 pathway. *Arterioscler Thromb Vasc Biol* 2013;33:565-71.

<http://dx.doi.org/10.1016/j.jaci.2017.02.030>

METHODS

Mice

All experiments were approved by the University of Tokyo's Institutional Animal Care and Use Committee. Male C57BL/6J-*Hpgds*^{-/-} mice,^{E1} C57BL/6J-*Crth2*^{-/-} mice,^{E2} C57BL/6J-*Mcpt5-Cre* mice,^{E3} FBV-H-PGDS transgenic mice,^{E4} and the age-matched (7–8 weeks old) control mice were generated and bred, as previously described. C57BL/6J-*Hpgds*^{fl/fl} mice were generated by using gene-targeting methods, and C57BL/6J-*Dp*^{-/-} mice were generated by means of offset-nicking of the CRISPR/Cas system^{E5} and backcrossed and used for the following experiments (Fig E7). C57BL/6J-*Hpgds*^{fl/fl} mice were generated as follows. To generate a targeting vector, genomic fragments of the *HPGDS* locus were obtained from the RP23-385O12 BAC clone (BACPAC Resources). A 9.5-kb region containing exons 2 and 3 of the *HPGDS* gene was flanked by loxP sites. Targeted embryonic stem clones were microinjected into 8-cell stage embryos of ICR strain mice, and injected embryos were transferred into pseudopregnant ICR females. The resulting chimeras were bred with C57BL/6 mice, and heterozygous offspring were identified by means of PCR with primers (F2, 5'-ACAG AGCTTAAC ACTGAGACGCAGCTGT-3'; R2, 5'-GCTTGAAAGGGAAG CTTGAAGGTGGT-3'; and neoR3, 5'-CCTACATTTTGAATGGAAGGAT TGGAGCT-3') yielding 320-bp products for the WT allele and 450-bp products for the targeted allele, respectively. C57BL/6J-*Dp*^{-/-} mice were generated by offset-nicking of CRISPR/Cas system by using 4 gRNAs (5'-target, TGAGGTAAGGAAACAGGGGGGGG and CATTGCAGCGATCTAAGAT TTGG; 3'-target, GTTCCCACAGTTCTTGCTGAGG and GAACTGTG GGAAGTGTGGGAGG). Genotyping of the obtained pups were performed by means of genomic PCR with the primers (forward, 5'-TCAAATCAAC ATGGAACTTGC-3'; reverse, 5'-GGAACCCAAGTGAGTAGGATTC-3') and direct sequencing and a pup in which the DP ORF in exon 1 deleted was backcrossed and used for following experiments.

Reagents

The following reagents were obtained from the indicated suppliers: Evans blue, naphthol AS-D chloroacetate, anti-DNP mAb produced in the mouse (clone: SPE-7), C48/80, Cy5.5 conjugated anti-mouse α -SMA, Alexa Fluor 568-labeled anti-rabbit IgG, and Alexa Fluor 647-labeled anti-rat IgG (Sigma-Aldrich, St Louis Mo); formamide, N-N dimethyl formamide, histamine dihydrochloride, sodium nitrate, diphenhydramine, and acetonitrile (Wako, Osaka, Japan); L-NAME (Enzo Life Sciences, Farmingdale, NY); BSA, 2,4-dinitrophenylated (Calbiochem, San Diego, Calif); and PGD₁, PGD₂, PGD₂-d₄, BW245C, 13,14-dihydro-15-keto PGD₂, and rabbit anti-HPGDS polyclonal antibody (Cayman Chemicals, Ann Arbor, Mich). Rat anti-PECAM-1 (CD31) mAb (Biocare Medical, Pacheco, Calif), fluorescein isothiocyanate-labeled avidin (Thermo Fisher Scientific, Waltham, Mass), and New Fuchsin (MP Biomedicals, Santa Ana, Calif).

Measurement of vascular hyperpermeability

Mice were sensitized by means of intradermal injection of anti-DNP IgE antibody (30 ng in 20 μ L of saline) in the ear. After 24 hours, DNP-BSA (60 μ g in 100 μ L of saline) was injected intravenously. In the other experiments C48/80 (100 ng in 20 μ L of saline) was challenged by means of intradermal injection in the ear or dorsal back skin. The H₁ blocker diphenhydramine (2.5 μ g in 20 μ L of saline) or L-NAME (10 or 30 μ g in 20 μ L of saline) was pretreated 15 minutes before C48/80 challenge, and PGD₁, PGD₂ (1 μ g in 20 μ L of saline), and the DP agonist BW245C (500 ng in 20 μ L of saline) were pretreated 5 minutes before C48/80 challenge. To assess the vascular permeability, Evans blue dye (50 mg/kg in 100 μ L of saline) was intravenously injected 5 minutes after intradermal anaphylactic stimulations. Thirty minutes after the injection, the ear or skin (6-mm square) was dissected, dried in a constant-temperature oven, and weighed. Any extravasated Evans blue was extracted in formamide for 48 hours and quantified spectrophotometrically at a wavelength of 610 nm.

Blood pressure measurement

Under isoflurane (1%)-induced anesthesia, a heparinized saline-filled catheter connected to a pressure transducer (Utah Medical Products, Midvale, Utah) was inserted into the left femoral artery. After basal blood pressure was stabilized, C48/80 (1 mg/kg in 100 μ L of saline) was administered into the jugular vein. In some experiments BW245C (1 ng/kg in 100 μ L of saline) was intraperitoneally injected in naive WT mice. The captured signal was transformed with PowerLab 8/35 (ADInstruments, Colorado Springs, Colo) into digital data and recorded with LabChart 7 (ADInstruments, Colorado Springs, Colo) every 5 minutes for 60 minutes. Data were expressed as millimeters of mercury.

Body temperature measurement

Mice were sensitized by means of intravenous injection of anti-DNP IgE (10 μ g in 20 μ L of saline) and challenged 24 hours later by means of intravenous injection of DNP-BSA (500 μ g in 100 μ L of saline). In the other experiments C48/80 (2 mg/kg in 100 μ L of saline) was intravenously injected. The H₁ blocker diphenhydramine (5 mg/kg in 100 μ L of saline) or L-NAME (200 mg/kg in 100 μ L of saline) was pretreated for 30 minutes and the DP agonist BW245C (1 ng/kg in 100 μ L of saline) was pretreated 5 minutes before C48/80 administration. Rectal temperature was measured every 5 minutes for 90 minutes by with microprobe thermometer (BAT-12; Physitemp, Clifton, NJ) in the conscious state.

Hematocrit, histamine, and PGD₂ measurement

Blood samples were obtained 5 minutes after intravenous administration of C48/80 (2 mg/kg) to determine hematocrit and plasma histamine levels. For PGD₂ measurement, ear samples were obtained 10 minutes after intradermal injection of C48/80 (100 ng). Histamine or PGD₂ levels were measured by using liquid chromatography–tandem mass spectrometry.

In preliminary experiments we measured plasma histamine levels after intravenous administration of C48/80 (2 mg/kg) at 0, 5, 10, 15, 30, and 60 minutes and found its highest level at 5 minutes. Ten microliters of plasma samples immediately mixed with 50 μ L of acetonitrile and precipitated extra proteins. After centrifugation (12,000g at 4°C for 5 minutes), the collected 50 μ L of supernatant was diluted into 365 μ L of distilled water. Ears were homogenized with an amalgam mixer (MM 300; Retsch, Haan, Germany) and mixed with 1 mL of ethanol containing 0.02% HCl (pH 2.0). After centrifugation at 500g for 20 minutes, supernatants were diluted with deionized water to 15% ethanol solutions, and PGD₂-d₄ (100 ng/mL, internal standard) was then added. Solutions were applied to Sep-Pak Vac 3 cc cartridges (Waters, Milford, Mass), and the cartridges were washed with n-hexane. The analyte and internal standard were eluted from the cartridge with ethyl acetate. The eluate was dried, and the residue was reconstituted in 10% (vol/vol) acetonitrile.

The sample solutions were then introduced into an LCMS-8030 system (Shimadzu, Columbia, Md) equipped with an electrospray (TurboSpray) interface. The liquid chromatography separation was conducted with a Inertsil ODS-3 column (GL Sciences, Torrance, Calif) by using a mobile phase consisting of 0.02% (vol/vol) acetic acid (solvent A) and acetonitrile (solvent B). The following gradient was used at a flow rate of 400 μ L/min: initial (2 minutes) at 95:5 (solvent A/solvent B), 10 minutes at 80:20, 18 minutes at 35:65, and 23 minutes at 10:90. Liquid chromatography–tandem mass spectrometry was operated in the positive and negative ion modes. For analysis, the monitored transition for histamine was m/z 111 $\rightarrow$ 95, that for PGD₂ was m/z 351 $\rightarrow$ 271, and that for PGD₂-d₄ was m/z 355 $\rightarrow$ 319. Histamine or PGD₂ was identified based on elution time: 0.95 or 14.3 minutes, respectively.

Whole-mount staining

After perfusion fixation with 4% paraformaldehyde, the ears were dissected and separated into dorsal and ventral flaps. The dorsal flaps were again fixed with 4% paraformaldehyde and incubated in PBS containing 0.5% Triton X-100 and 5% normal goat serum for 1 hour at room temperature. Then the samples were stained with the following primary antibodies overnight at

4°C: rabbit anti-HPGDS and rat anti-PECAM-1 mAb. After washing for 3 times, the sample was incubated with the following antibodies for 3 hours at room temperature: Alexa Fluor 568-labeled anti-rabbit IgG, Alexa Fluor-labeled 647 anti-rat IgG, and Cy5.5-conjugated anti-mouse α -SMA. Mast cells were labeled with fluorescein isothiocyanate-conjugated mouse anti-avidin. Images were captured with LSM 700 laser scanning microscope (Zeiss, Oberkochen, Germany).

Statistical analyses

Data were analyzed with Prism 6.0 software (GraphPad Software, La Jolla, Calif). Blood pressure and temperature curves were analyzed by means of repeated-measures 2-way ANOVA with the Bonferroni posttest. Multiple-group comparisons were analyzed by using a 1-way ANOVA with the Tukey posttest.

REFERENCES

- E1. Mohri I, Taniike M, Taniguchi H, Kanekiyo T, Aritake K, Inui T, et al. Prostaglandin D2-mediated microglia/astrocyte interaction enhances astrogliosis and demyelination in twitcher. *J Neurosci* 2006;26:4383-93.
- E2. Satoh T, Moroi R, Aritake K, Urade Y, Kanai Y, Sumi K, et al. Prostaglandin D2 plays an essential role in chronic allergic inflammation of the skin via CRTH2 receptor. *J Immunol* 2006;177:2621-9.
- E3. Scholten J, Hartmann K, Gerbaulet A, Krieg T, Muller W, Testa G, et al. Mast cell-specific Cre/loxP-mediated recombination in vivo. *Transgenic Res* 2008;17:307-15.
- E4. Fujitani Y, Aritake K, Kanaoka Y, Goto T, Takahashi N, Fujimori K, et al. Pronounced adipogenesis and increased insulin sensitivity caused by overproduction of prostaglandin D2 in vivo. *FEBS J* 2010;277:1410-9.
- E5. Fujii W, Onuma A, Sugiura K, Naito K. Efficient generation of genome-modified mice via offset-nicking by CRISPR/Cas system. *Biochem Biophys Res Commun* 2014;445:791-4.

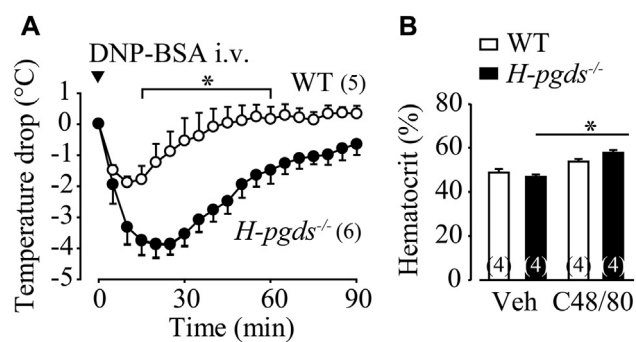


FIG E1. HPGDS deficiency enhances systemic anaphylaxis. **A**, Antigen/antibody-induced body temperature decrease. **B**, Hematocrit level 10 minutes after intravenous (*i.v.*) C48/80 administration. Numbers of analyzed animals are given in parentheses. Data were obtained in 3 independent experiments and are represented as means $\pm$ SEMs. * $P < .05$.

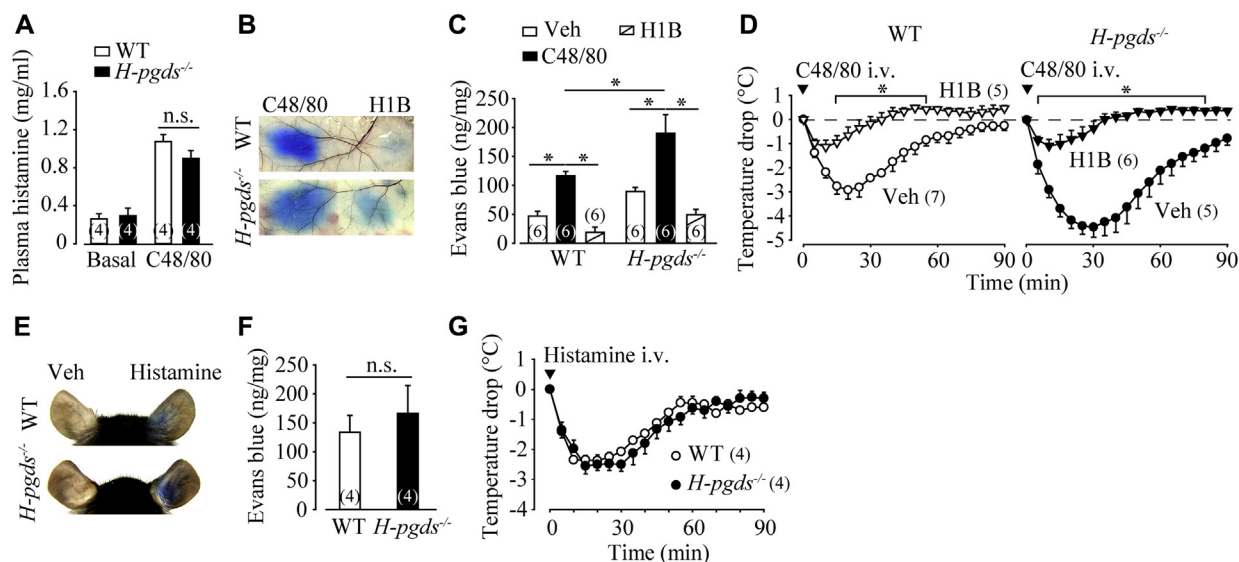


FIG E2. C48/80-induced anaphylaxis depends on the histamine H₁ receptor in WT and *Hpgds*^{-/-}. **A**, Plasma histamine levels 5 minutes after intravenous (*i.v.*) C48/80 administration. **B-D**, Effect of the H₁ blocker on C48/80-induced (Fig E2, **B** and **C**) dye extravasation and hypothermia (Fig E2, **D**). **E-G**, Histamine-induced dye extravasation (Fig E2, **E** and **F**) and hypothermia (Fig E2, **G**). Numbers of analyzed animals are given in parentheses. Data were obtained in 2 to 3 independent experiments and are represented as means ± SEMs. **P* < .05.

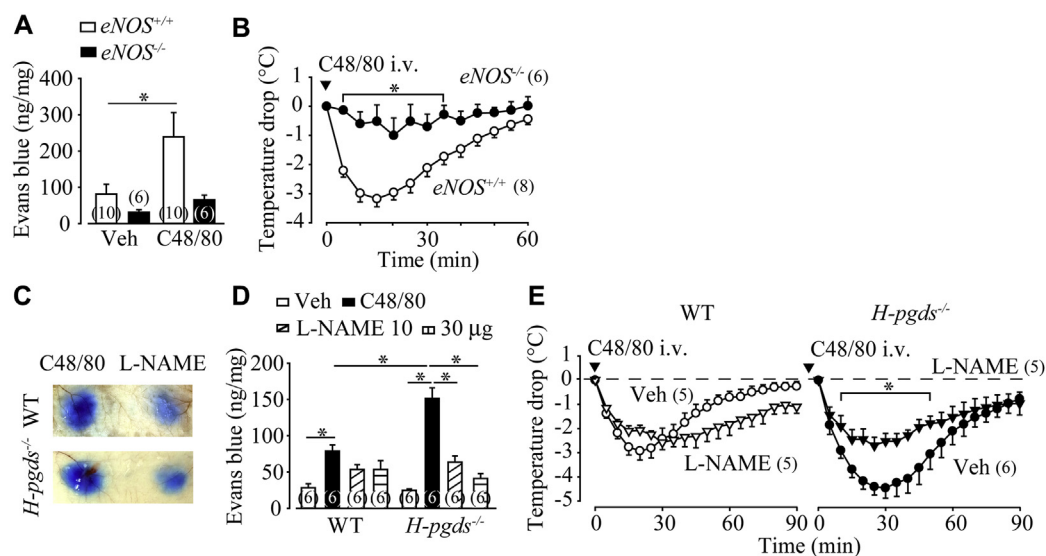


FIG E3. C48/80-induced anaphylaxis depends on endothelial nitric oxide synthase (*eNOS*)-NO in WT and *Hpgds*^{-/-} mice. **A** and **B**, C48/80-induced dye extravasation (Fig E3, A) and hypothermia (Fig E3, B). **C-E**, Effect of L-NAME on C48/80-induced dye extravasation (Fig E3, C and D) and hypothermia (Fig E3, E). Numbers of analyzed animals are given in parentheses. Data were obtained in 2 to 3 independent experiments and are represented as means $\pm$ SEMs. **P* < .05. i.v., Intravenous; n.s., not significant.

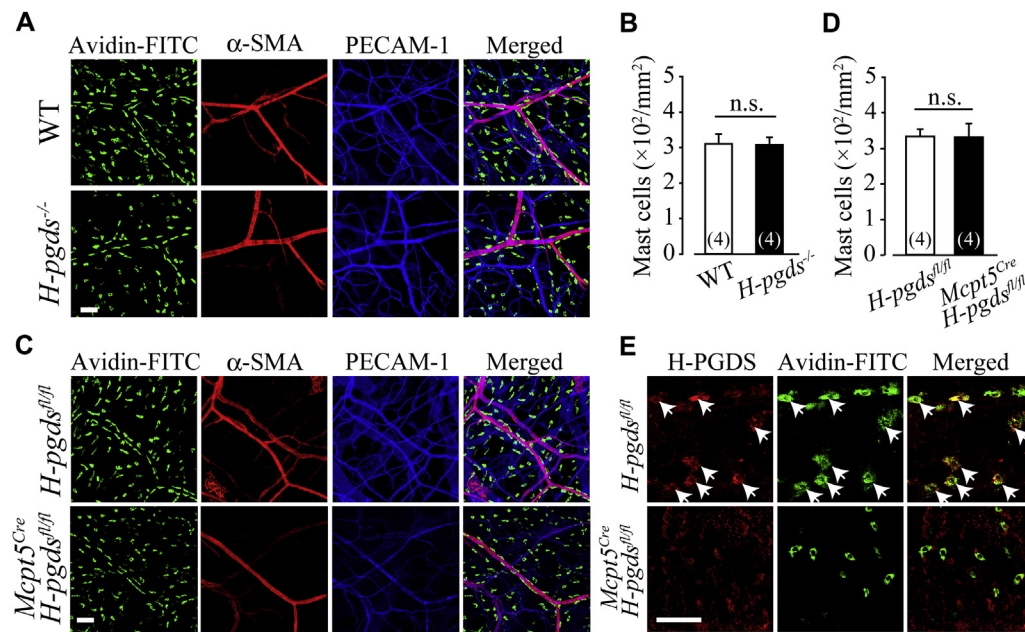


FIG E4. Perivascular mast cells express HPGDS. **A** and **C**, Whole-mount immunostaining of an ear section stained with SMA (red) and endothelial cells (blue). Scale bar = 100 μm . **B** and **D**, Mean number of mast cells per square millimeter in the ear. **E**, Whole-mount immunostaining of an ear section stained with HPGDS (red). Mast cells were labeled with avidin-FITC (green). Scale bar = 50 μm . Numbers of analyzed animals are given in parentheses. Data were obtained in 2 to 3 independent experiments. n.s., Not significant.

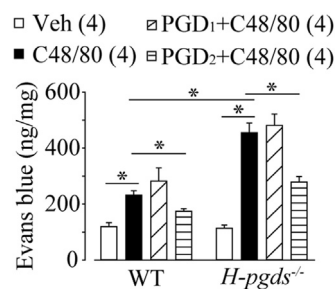


FIG E5. PGD₂ but not PGD₁ inhibits C48/80-induced vascular hyperpermeability. The effect of PGD₁ (1 μg) or PGD₂ (1 μg) on C48/80-induced dye extravasation on back skin. Numbers of analyzed animals are given in parentheses. Data were obtained in 2 independent experiments and are represented as means ± SEMs. **P* < .05.

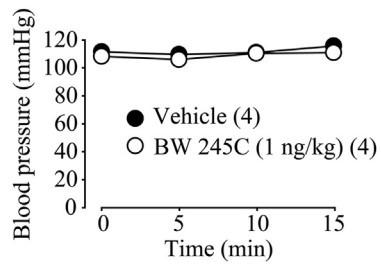


FIG E6. DP activation does not induce any decrease in blood pressure. The trace of the blood pressure in vehicle- or DP agonist (BW245C: 1 ng/kg)-treated WT mice is shown. Numbers of analyzed animals are given in parentheses. Data were obtained in 2 independent experiments.

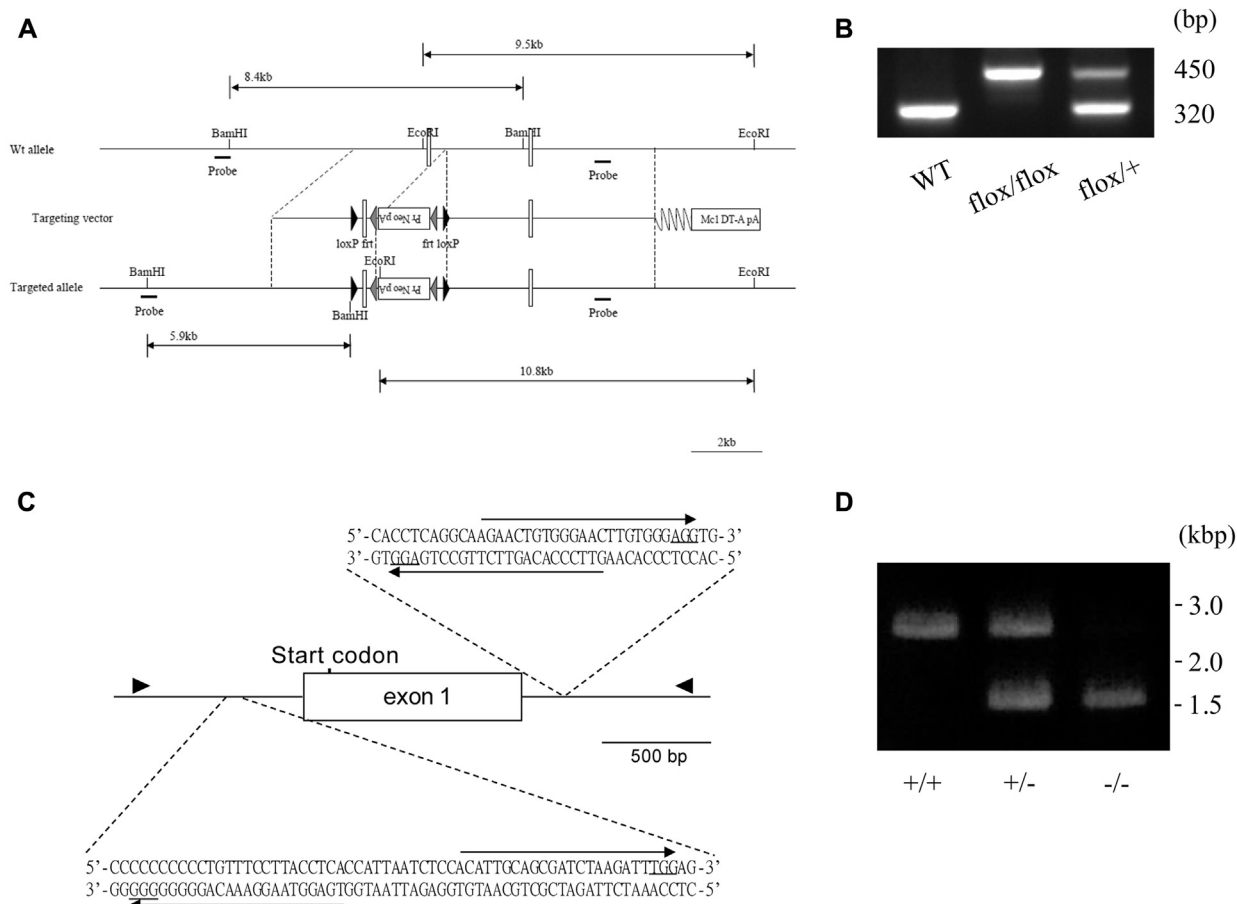


FIG E7. Generation of *Hpgds*^{fl/fl} and *Dp*^{-/-} mice. **A**, Strategy for generating the *HPGDS* targeting vector and *HPGDS* floxed allele. **B**, Genotype of *Hpgds*^{fl/fl} mice was identified by means of PCR. **C**, Schematic illustration of DP gene structure and sequences around target loci. Target sequences and PAM and PCR primer loci are indicated by *arrows*, *underlining*, and *arrowheads*, respectively. **D**, Genotype of *Dp*^{-/-} mice was identified by means of PCR.