

Dynamin-related protein 1 differentially regulates FcεRI- and substance P-induced mast cell activation



Ying Wang, MD, PhD,^a Mang Yu, MD, PhD,^a Kazufumi Matsushita, PhD,^{a,b} Chen Liu, MD,^{a,b} Naotada Ishihara, PhD,^d Masatoshi Nomura, MD,^e Mindy Tsai, DMSc,^{a,b} and Stephen J. Galli, MD^{a,b,c} *Stanford, Calif; and Osaka and Kurume, Japan*

Background: The mitochondrial fission protein dynamin-related protein 1 (Drp1) has been suggested to regulate mast cell (MC) activation by certain stimuli *in vitro*, but its functions in MCs activated by various stimuli *in vivo* have not yet been examined. **Objective:** We sought to analyze Drp1 function in both mouse and human MCs.

Methods: We used human peripheral blood–derived cultured MCs and 2 genetic mouse models in which MCs were depleted of Drp1: *Drp1^{fl/fl}Mcpt5cre^{+/-}* mice and *Drp1^{fl/fl}Cpa3cre^{+/-}* mice.

Results: In mice, Drp1 depletion enhanced FcεRI-induced MC activation while suppressing substance P–stimulated MC activation *in vitro* and *in vivo*. This was also true in human peripheral blood–derived cultured MCs *in vitro* after pharmacologic inhibition of Drp1.

Conclusion: Drp1 differentially regulates MC activation by various stimuli. Promoting Drp1 activation might therefore represent a novel therapy for suppressing IgE-dependent MC activation. Further, inhibiting Drp1 activation might mitigate other MC-dependent responses, such as those induced by substance P. (J Allergy Clin Immunol 2022;150:1228–31.)

Key words: Mast cell, FcεRI-dependent activation, Drp1, substance P

Abbreviations used

Av.A488: Alexa Fluor 488–coupled avidin
β-hex: β-Hexosaminidase
BM: Bone marrow
BMCMC: BM-derived cultured MC
Drp1: Dynamin-related protein 1
GPCR: G protein–coupled receptor
MC: Mast cell
PBCMC: Human primary peripheral blood–derived cultured MC
PCA: Passive cutaneous anaphylaxis
SP: Substance P

INTRODUCTION

Mast cell (MC) activation via cross-linking of the high-affinity IgE receptor FcεRI can contribute to allergic conditions such as asthma, food allergy, and atopic dermatitis.¹ This process is initiated when specific antigens cross-link FcεRI-bound IgE. However, stimuli that can activate MCs via FcεRI-independent mechanisms can also contribute to inflammatory processes, including cationic substances like the neuropeptide substance P (SP).²

However, factors that can regulate FcεRI-dependent and -independent activation of MCs are not fully understood. A recent study has shown that mitochondrial fission and translocation occurs during activation of cultured MCs *in vitro*.³ Moreover, expression of mRNA of dynamin-related protein 1 (Drp1), an essential molecule for mitochondrial fission, has been reported to increase in skin MCs from patients with atopic dermatitis, suggesting a positive correlation between Drp1 and MC activation in atopic dermatitis patients.³ Notably, however, the specific role of Drp1 in MC activation (eg, by IgE or SP) *in vivo* has not yet been examined.

RESULTS AND DISCUSSION

To investigate the role of Drp1 in MC activation *in vivo*, we generated *Drp1^{fl/fl}Mcpt5cre^{+/-}* mice by crossing *Drp1^{fl/fl}* mice⁴ with *Mcpt5cre^{+/-}* mice.⁵ Because *Mcpt5* is dominantly expressed in connective tissue–type MCs,⁵ we expected to delete Drp1 in connective tissue MCs with *Mcpt5cre*. We first confirmed that *Mcpt5cre* is expressed in connective MCs in the ear pinna on the basis of the observation that the ear pinna dermis was depleted of MCs in *Mcpt5cre^{+/-} R-DTA* mice, in which diphtheria toxin A kills *Mcpt5*-expressing cells (see Fig E1, A, in this article's Online Repository at www.jacionline.org). Depletion of MCs in the

From ^athe Department of Pathology, ^bthe Sean N. Parker Center for Allergy and Asthma Research, and ^cthe Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford; ^dthe Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka; and ^ethe Division of Endocrinology and Metabolism, Department of Internal Medicine, Kurume University School of Medicine, Kurume.

The first 3 authors contributed equally to this article, and all should be considered first author.

Supported by a National Institutes of Health (NIH) T32 training award to Y.W. (T32 AI 7290-33), an award from MSD Life Science Foundation, Public Interest Incorporated Foundation (Japan) to K.M., NIH grants (National Institute of Allergy and Infectious Diseases [NIAID] R01 AI132494, NIAID U19AI104209, National Institute of Arthritis and Musculoskeletal and Skin Diseases [NIAMS] R01 AR067145), and the United States–Israel Binational Science Foundation (grant 2017182) to S.J.G.

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

Received for publication December 16, 2021; revised April 14, 2022; accepted for publication April 21, 2022.

Available online May 11, 2022.

Corresponding author: Stephen J. Galli, MD, Center for Clinical Sciences Research, 269 Campus Drive, Room 3255b, Stanford, CA 94305-5176. E-mail: sgalli@stanford.edu.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/\$36.00

© 2022 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaci.2022.04.028>

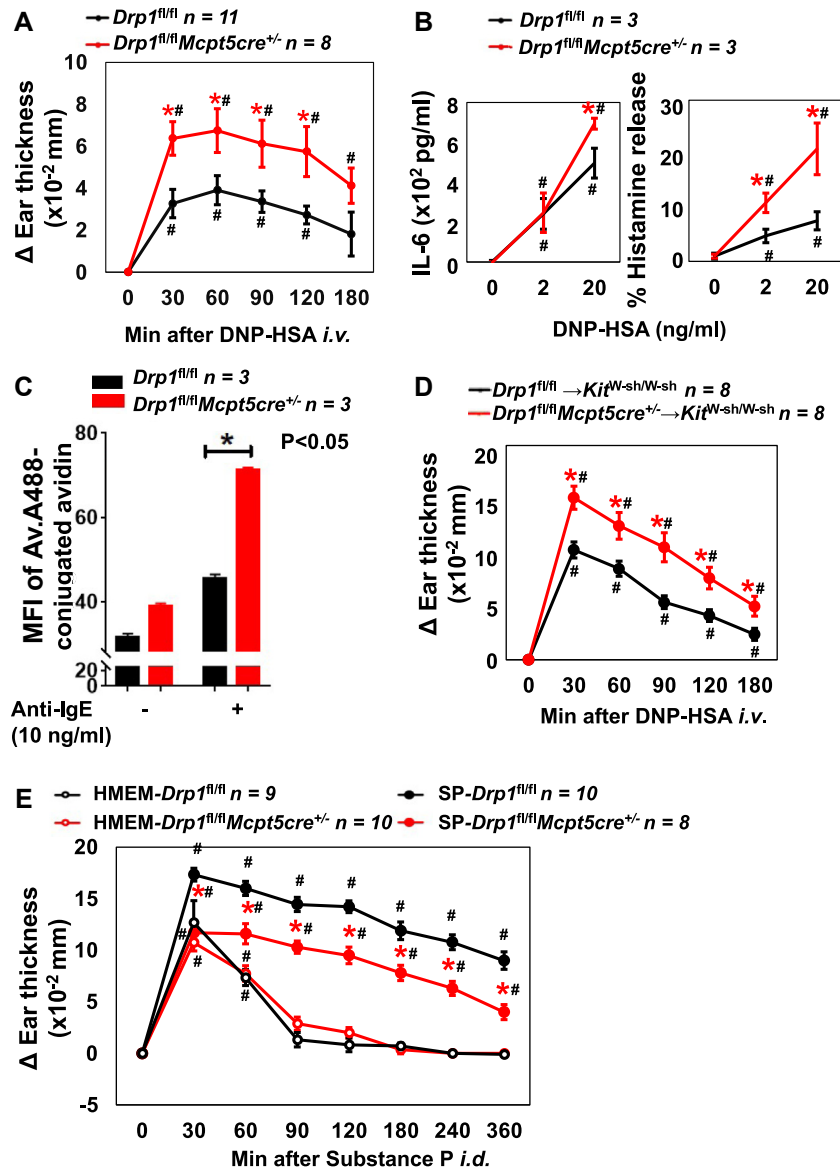


FIG 1. Drp1 depletion enhances FcεRI-induced MC activation in mice. (A) PCA reactions (female mice). (B) IL-6 and percentage histamine release from IgE-sensitized, DNP-HSA-stimulated BMCMCs. (C) IgE activation of BMCMCs (mean fluorescence intensity [MFI] of Av.A488). **P* < .05 (D) PCA reactions (male mice). (E) SP-induced cutaneous inflammation. Error bars indicate SEMs. **P* < .05 vs other group; #*P* < .05 vs 0 DNP-HSA. DNP-HSA, Dinitrophenol (30–40 mol/mol)-conjugated human serum albumin.

ear pinna virtually abolished expression of the passive cutaneous anaphylaxis (PCA) reaction (Fig E1, B). These observations are in accord with the previous finding that in *Mcpt5-Cre ROSA26-EYFP* double-transgenic mice, EYFP is specifically expressed in connective MCs (peritoneal and skin MCs) but not in other types of bone marrow (BM)-derived cells.⁵

Drp1^{fl/fl}Mcpt5cre^{+/-} mice were born in the expected Mendelian ratio and did not show any gross developmental defects. The MC population in the peritoneal cavity and the total numbers of MCs in the skin were similar in *Drp1^{fl/fl}Mcpt5cre^{+/-}* and *Drp1^{fl/fl}* animals (see Fig E2, A, in the Online Repository at www.jacionline.org). However, *Drp1^{fl/fl}Mcpt5cre^{+/-}* mice displayed enhanced PCA reactions in the ear pinna (Fig 1, A). These

phenotypes were not due to the expression of *Mcpt5cre* alone (Fig E1, C).

We then generated IL-3–dependent BM-derived cultured MCs (BMCMCs) by culturing BM cells in IL-3–conditioned media.^{6,7} Western blot analysis validated the notion that the expression of Drp1 in BMCMCs derived from *Drp1^{fl/fl}Mcpt5cre^{+/-}* mice is substantially reduced compared to BMCMCs derived from *Drp1^{fl/fl}* mice (Fig E2, B). *Drp1^{fl/fl}Mcpt5cre^{+/-}* BMCMCs released significantly more IL-6 and more histamine than *Drp1^{fl/fl}* BMCMCs on FcεRI activation (Fig 1, B). Notably, the enhanced histamine release of *Drp1^{fl/fl}Mcpt5cre^{+/-}* BMCMCs was not due to an increase in total intracellular histamine content (Fig E2, C).

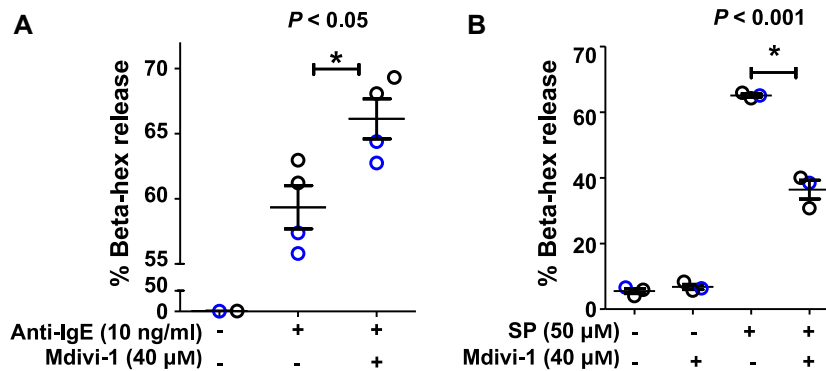


FIG 2. Drp1 depletion enhances FcεRI-induced activation but inhibits SP activation in human MCs *in vitro*. β-Hex release from human PBCMCs activated with anti-IgE (A) or PBCMCs activated with SP (B), with or without the incubation with Mdivi-1, a selective Drp1 inhibitor. PBCMCs were from 2 donors; 2 experiments were performed per donor. Error bars indicate SEMs.

To further characterize MC degranulation *in vitro*, we used a soluble fluorochrome-labeled avidin: Alexa Fluor 488-coupled avidin (Av.A488).^{8,9} During MC degranulation, secretory granule membranes fuse with the plasma membrane, and the granule matrix is externalized and rapidly bound by fluorescent avidin, allowing monitoring of degranulation dynamics by flow cytometry. The level of degranulation can be quantified as the mean fluorescence intensity of Av.A488 on the cell surface and the percentage of Av.A488⁺ cells. Using this assay, we confirmed that *Drp1*^{fl/fl}*Mcpt5cre*^{+/-} BMCs showed enhanced degranulation on FcεRI-induced activation (Fig 1, C, and see Fig E2, D). Furthermore, MC-deficient *Kit*^{W-sh/W-sh} mice engrafted with *Drp1*^{fl/fl}*Mcpt5cre*^{+/-} MCs in the ear pinna exhibited stronger PCA reactions than did *Kit*^{W-sh/W-sh} animals engrafted with *Drp1*^{fl/fl} MCs (Fig 1, D), although the number of engrafted MCs was not significantly different between the 2 groups (Fig E2, E). Taken together, these findings show that suppression of Drp1 in MCs increases the intensity of PCA *in vivo* and also enhances FcεRI-induced MC degranulation and histamine and cytokine release *in vitro*.

To confirm these findings, we isolated BM cells and generated BMCs from *Drp1*^{fl/fl}*Cpa3cre*^{+/-} mice. *Cpa3* is ubiquitously expressed in tissue MCs. After 6 weeks in culture, both *Drp1*^{fl/fl} and *Drp1*^{fl/fl}*Cpa3cre*^{+/-} MCs were >95% FcεRIα and c-Kit double-positive MCs (see Fig E3, A, in the Online Repository at www.jacionline.org). As expected, the expression of Drp1 protein in *Drp1*^{fl/fl}*Cpa3cre*^{+/-} MC was reduced by >90% in the total cell lysate (Fig E3, B). Similar to *Drp1*^{fl/fl}*Mcpt5cre*^{+/-} MCs, *Drp1*^{fl/fl}*Cpa3cre*^{+/-} MCs released more IL-6 and histamine on FcεRI stimulation than *Drp1*^{fl/fl} MCs (Fig E3, C). This was not associated with increased expression of FcεRIα or c-Kit on the cell surface of *Drp1*^{fl/fl}*Cpa3cre*^{+/-} MCs (Fig E3, F). The calcium ionophore A23187 (1 μmol) can elicit sustained elevations of intracellular calcium,⁸ mimicking intracellular calcium dynamics during FcεRI-dependent MC activation.⁹ We found that A23187-induced IL-6 release was increased in *Drp1*^{fl/fl}*Mcpt5cre*^{+/-} MCs compared to *Drp1*^{fl/fl} MCs (Fig E3, D), suggesting that calcium functions upstream of Drp1 in the cascade of FcεRI-dependent MC activation.

Most importantly, engraftment of *Drp1*^{fl/fl}*Cpa3cre*^{+/-} MCs into the ear pinnae of MC-deficient *Kit*^{W-sh/W-sh} mice primed them to

express a stronger PCA reaction than was observed in the *Kit*^{W-sh/W-sh} animals engrafted with *Drp1*^{fl/fl} MCs (Fig E3, E). Such stronger PCA reactions were not due to increased numbers of MCs in the ear pinna after engraftment (Fig E3, G). These findings in *Drp1*^{fl/fl}*Cpa3cre*^{+/-} mice thus are consistent with those from *Drp1*^{fl/fl}*Mcpt5cre*^{+/-} mice in showing that depletion of Drp1 enhances FcεRI-mediated MC activation both *in vivo* and *in vitro*.

We also investigated the potential role of Drp1 in one example of FcεRI-independent MC activation: the pseudo-allergic/neurogenic route of MC activation induced by SP. In contrast to FcεRI-induced MC activation, we found that *Drp1*^{fl/fl}*Mcpt5cre*^{+/-} mice exhibited reduced SP-induced ear swelling compared to control *Drp1*^{fl/fl} mice (Fig 1, E). This finding shows that Drp1 can enhance SP-induced MC activation *in vivo*.

Finally, we tested the effect of Drp1 inhibition during activation of primary human peripheral blood-derived cultured MCs PBCMCs *in vitro*. Pretreatment of PBCMCs with 40 μmol of the Drp1-specific inhibitor Mdivi-1 promoted FcεRI-mediated β-hexosaminidase (β-hex) release (Fig 2, A). Interestingly, the same dose of Mdivi-1 did not increase, but instead suppressed, SP-mediated human MC β-hex release (Fig 2, B). This result is consistent with the report from Zhang et al³ that Mdivi-1 suppressed SP-induced TNF-α and β-hex release in the human MC line LAD2 cells.

Using both human and mouse MC models, we found that Drp1 can suppress FcεRI-mediated MC activation while enhancing SP-induced MC activation. Thus, Drp1 can differentially influence MC activation during the cells' stimulation with distinct agonists.

Such differential effects of Drp1 in MCs activated by various stimuli (SP vs IgE) might be explained by the diverse effects of Drp1 in signaling pathways downstream of individual stimuli.¹⁰⁻¹⁴ Although Drp1 directly mediates mitochondrial outer membrane fusion, more recent studies have reported the pleiotropic effects of Drp1 in regulating intracellular calcium compartmentation, organellar interactions, and functions.^{11,12,14} Indeed, a recent study from our lab has shown that IgE-FcεRI-dependent cutaneous reactions are long lasting; in contrast, a pseudo-allergic reaction (ie, MRGPRX2-dependent MC activation induced by SP) appears to be more transient.⁹ An apparent difference between the 2 activation pathways has been described: SP

induces a rapid and transient secretion of relatively small individual spherical granules, while FcεRI-dependent activation causes a delayed secretion of larger, more heterogeneous granules, likely as a result of granule fusion.⁹ Therefore, one plausible explanation of our observations is that Drp1 differentially regulates MC granule fusion and secretion downstream of SP stimulation versus ligation of FcεRI.

Besides Drp1, the differential regulation of distinct stimuli-induced MC degranulation has been reported. For example, G protein-coupled receptor (GPCR) kinase 2 (GRK2) inhibits human MC degranulation in response to GPCR-dependent activation by C3a while augmenting activation in response to IgE cross-linking.^{15,16} Such differential MC activation is associated with differential regulation of intracellular calcium dynamics. It will be interesting to determine whether the opposite effects of Drp1 in the MC's response to SP stimulation versus ligation of FcεRI is dependent on GRK2, on other GPCR-associated proteins, on calcium dynamics, or on some combination of these.

In conclusion, our work shows that Drp1 differentially regulates MC activation by 2 stimuli, IgE+ antigen and SP. Indeed, Drp1 downregulates FcεRI-induced MC activation but upregulates SP-induced MC activation. These findings suggest that promoting Drp1 activation might represent a novel therapy for suppressing IgE-dependent MC activation, whereas inhibiting Drp1 activation may mitigate other MC-dependent responses, such as those induced by SP.

Key messages

- Inhibition of Drp1 enhances FcεRI-dependent MC activation but reduces SP-induced MC activation in mice.
- Such differential regulation of MC activation also can be observed in human PBCMCs, indicating a potential context-dependent role of Drp1 in MC activation in human diseases.

REFERENCES

1. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012;18:693-704.
2. Yano H, Wershil BK, Arizono N, Galli SJ. Substance P-induced augmentation of cutaneous vascular permeability and granulocyte infiltration in mice is mast cell dependent. *J Clin Invest* 1989;84:1276-86.
3. Zhang B, Alysandratos KD, Angelidou A, Asadi S, Sismanopoulos N, Delivanis DA, et al. Human mast cell degranulation and preformed TNF secretion require mitochondrial translocation to exocytosis sites: relevance to atopic dermatitis. *J Allergy Clin Immunol* 2011;127:1522-31.e8.
4. Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, et al. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat Cell Biol* 2009;11:958-66.
5. 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.
6. Tam SY, Tsai M, Snouwaert JN, Kalesnikoff J, Scherrer D, Nakae S, et al. Rab-GEF1 is a negative regulator of mast cell activation and skin inflammation. *Nat Immunol* 2004;5:844-52.
7. Yu M, Mukai K, Tsai M, Galli SJ. Thirdhand smoke component can exacerbate a mouse asthma model through mast cells. *J Allergy Clin Immunol* 2018;142:1618-27.e9.
8. Gullledge TV, Collette NM, Mackey E, Johnstone SE, Moazami Y, Todd DA, et al. Mast cell degranulation and calcium influx are inhibited by an *Echinacea purpurea* extract and the alkylamide dodeca-2E,4E-dienoic acid isobutylamide. *J Ethnopharmacol* 2018;212:166-74.
9. Gaudenzio N, Sibillano R, Marichal T, Starkl P, Reber LL, Cenac N, et al. Different activation signals induce distinct mast cell degranulation strategies. *J Clin Invest* 2016;126:3981-98.
10. Bulfone-Paus S, Nilsson G, Draber P, Blank U, Levi-Schaffer F. Positive and negative signals in mast cell activation. *Trends Immunol* 2017;38:657-67.
11. Li X, Yang L, Mao Z, Pan X, Zhao Y, Gu X, et al. Novel role of dynamin-related protein 1 in dynamics of ER-lipid droplets in adipose tissue. *FASEB J* 2020;34:8265-82.
12. Liu W, Ye C, Cheng Q, Zhang X, Yao L, Li Q, et al. Macrophage raptor deficiency-induced lysosome dysfunction exacerbates nonalcoholic steatohepatitis. *Cell Mol Gastroenterol Hepatol* 2019;7:211-31.
13. Kraus F, Roy K, Pucadyil TJ, Ryan MT. Function and regulation of the divisome for mitochondrial fission. *Nature* 2021;590(7844):57-66.
14. Rogers MA, Hutcheson JD, Okui T, Goettsch C, Singh SA, Halu A, et al. Dynamin-related protein 1 inhibition reduces hepatic PCSK9 secretion. *Cardiovasc Res* 2021;117:2340-53.
15. Guo Q, Subramanian H, Gupta K, Ali H. Regulation of C3a receptor signaling in human mast cells by G protein coupled receptor kinases. *PLoS One* 2011;6:e22559.
16. Subramanian H, Gupta K, Parameswaran N, Ali H. Regulation of FcεRI signaling in mast cells by G protein-coupled receptor kinase 2 and its RH domain. *J Biol Chem* 2014;289:20917-27.

METHODS

Mice

All mice were housed and bred in specific-pathogen-free animal facilities at Stanford University under a 12-hour light/12-hour dark cycle. The treatment of all the mice for these studies was in accord with institutional guidelines with review and was approved by Stanford's Institutional Animal Care and Use Committee. *Drp1^{fl/fl}* mice were kindly provided by Naotada Ishihara and Masatoshi Normura (Osaka University and Kurume University).^{E1} *Cpa3Cre⁺* transgenic mice were generated in the Galli lab, as described elsewhere.^{E2} *C57BL/6-Kit^{W-sh/W-sh}* mice were originally provided by Peter Besmer (Memorial Sloan-Kettering Cancer Center). We then backcrossed these mice to *C57BL/6J* mice for more than 11 generations.^{E3} *C57BL/6-Mcpt5Cre⁺* mice were provided by Axel Roers (University of Technology Dresden). *B6.129P2-Gt(ROSA)26Sor^{tm1(DTA)Lox/J} (R-DTA)* mice were obtained from The Jackson Laboratory (Bar Harbor, Me).

Passive cutaneous anaphylaxis

IgE-dependent PCA was induced in the ear pinna as described previously.^{E2} Briefly, mice under isoflurane anesthesia were sensitized passively with IgE by intradermal injection of 20 ng of dinitrophenol (DNP)-specific IgE (α -DNP clone 26, using cells kindly provided by Fu-Tong Liu [University of California, Davis] and D. H. Katz [LIDAK Pharmaceuticals]) in 20 μ L of Hanks minimum essential medium (HMEM)-piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) buffer (Sigma-Aldrich, St Louis, Mo) in the left ear pinna; mice received 20 μ L of vehicle intradermally in the right ear pinna as a control. The next day, mice were challenged through the retroorbital venous sinus with 100 μ g of DNP (30–40 mol/mol)-conjugated human serum albumin (DNP-HSA; Sigma) in 100 μ L of saline. Immediately before or at defined intervals after antigen challenge, ear thickness was measured with a dial thickness gauge (G-1A; Ozaki Manufacturing, Kobe City, Japan). Differences between left and right ear pinna were calculated (Δ ear thickness). Mice were humanely killed 6 hours after antigen challenge, and ear pinnae were collected for histologic analysis.

SP-dependent cutaneous inflammation

SP-induced cutaneous inflammation was induced in the ear pinna as described previously,^{E3,E4} with modifications. Briefly, the left ear pinna was injected intradermally with SP (1 nmol in 5 μ L HMEM solution containing 0.47 g/L PIPES) or intradermally with 5 μ L HMEM-PIPES (control). Immediately before or at defined intervals after antigen challenge, ear thickness was measured with a dial thickness gauge (G-1A; Ozaki Manufacturing). Differences between left and right ear pinna were calculated (Δ ear thickness).

MC culture, degranulation, and activation

Mouse BMCMCs were generated as previously described.^{E5} BM cells derived from 3-week-old female or male *Drp1^{fl/fl}* and *Drp1^{fl/fl}Cpa3Cre^{+/+}* mice, or from 4-week-old *Drp1^{fl/fl}* and *Drp1^{fl/fl}Mcpt5Cre^{+/+}* mice were cultured in 20% WEHI-3-conditioned media (TIB-68; American Type Culture Collection [ATCC], Manassas, Va) as a source of IL-3 for 6 weeks to generate cell populations that contain >95% immature MCs. Human PBCMCs were generated as previously described, with modification.^{E3} CD34⁺ peripheral blood cells were isolated from peripheral blood mononuclear cells using a Human CD34 Positive Selection Kit (EasySep 18086; STEMCELL Technologies, Vancouver, British Columbia, Canada). CD34⁺ cells were cultured and expanded in StemSpan Serum-Free Expansion Media (SFEM 09650; STEMCELL Technologies) supplemented with IL-6 (50 ng/mL) (200-06; PeproTech, London, United Kingdom) and stem cell factor (100 ng/mL) (300-07; PeproTech). PBCMCs were tested for purity and phenotype by flow cytometry (CD117, Fc ϵ RI α) and activation (β -hex release in response to Fc ϵ RI cross-linking) at 8 to 12 weeks.

Fc ϵ RI α -mediated BMCMC degranulation was performed as described elsewhere,^{E6} with modification. Hybridoma H1-DNP- ϵ 26, which produces IgE mAb to DNP, was used to generate ascites. MCs were sensitized with ascites diluted in medium at a concentration of 1 μ g/mL overnight at 37°C, washed with medium, and resuspended at 37°C with medium containing various concentrations of dinitrophenol (30–40 mol/mol)-conjugated human serum albumin

conjugated to each molecule of HSA (DNP-HSA; Sigma) for 30 minutes or 10 ng/mL DNP-HSA for the indicated times. In some experiments, unsensitized MCs were suspended in medium containing the indicated concentrations of calcium ionophore A23187 for 6 hours. Activation was stopped by immediately placing the cells on ice. For PBCMC activation, PBCMCs were preincubated with human IgE (1 μ g/mL) for 1 hour, then stimulated with 10 ng/mL anti-IgE for 30 minutes. For measuring histamine release, the total amount of histamine in the supernatant and cell lysate was determined by ELISA (IM2015; Beckman Coulter, Fullerton, Calif) after 6 hours. IL-6 release was quantified using a human IL-6 ELISA kit (EH2IL6; Thermo Fisher Scientific, Waltham, Mass) after 6 hours. The release of β -hex was measured on an ELISA reader with *p*-nitrophenyl-*N*-acetyl- β -D-glucosamine (Sigma), as described elsewhere.^{E6} Briefly, 1×10^5 cells were resuspended in 100 μ L Tyrode solution supplied with 1% glucose and 1% bovine serum albumin and HEPES (10 mmol) (Gibco, Thermo Fisher) in a V-shaped 96-well plate. Cells were then stimulated for 30 minutes at 37°C with 5% CO₂. At the end of the experiment, we collected supernatant and cell lysate (lysed in Tyrode solution + 0.5% Triton X-100), which were then analyzed by ELISA. Mdivi-1 (M0199), A23187 (C7522), and SP (S6883) were purchased from Sigma.

Engraftment of BMCMCs into *Kit^{W-sh/W-sh}* mice

Engraftment of BMCMCs into *Kit^{W-sh/W-sh}* mice was performed as described, with modification.^{E2} BMCMCs derived from female *Drp1^{fl/fl}* and *Drp1^{fl/fl}Cpa3Cre^{+/+}* (*C57BL/6J*) mice were transferred by intradermal injection (1×10^6 cells in 20 μ L of Dulbecco modified Eagle medium in a single injection into the left ear pinna, 20 μ L of Dulbecco modified Eagle medium alone into the right pinna) into 4-week-old male *Kit^{W-sh/W-sh}* mice ($n = 11$ *Drp1^{fl/fl}* $\rightarrow$ *Kit^{W-sh/W-sh}* and 8 *Drp1^{fl/fl}Cpa3Cre^{+/+}* $\rightarrow$ *Kit^{W-sh/W-sh}* mice). In another experiment, BMCMCs derived from female *Drp1^{fl/fl}* and *Drp1^{fl/fl}Mcpt5Cre^{+/+}* (*C57BL/6J*) mice were transferred into 4-week-old male *Kit^{W-sh/W-sh}* mice ($n = 8$ *Drp1^{fl/fl}* $\rightarrow$ *Kit^{W-sh/W-sh}* and 8 *Drp1^{fl/fl}Mcpt5Cre^{+/+}* $\rightarrow$ *Kit^{W-sh/W-sh}*). Experiments were initiated 8 weeks after injection of the BMCMCs.

Flow cytometry analysis

Flow cytometry analysis was performed with a BD FACSCanto II analyzer (Becton Dickinson, San Diego, Calif). Cells were suspended in phosphate-buffered saline + 0.5% bovine serum albumin + 0.5 mmol EDTA, then stained with the following antibodies or dyes for 30 minutes on ice: human CD117 (clone 104D2, 1:100), human Fc ϵ RI α (clone AER-37, 1:100), and 1 μ g/mL Av.A488.

Tissue sectioning and toluidine blue staining

Tissue specimens were fixed with 10% neutral buffered formalin and embedded in paraffin. Four-micron sections were stained with 0.1% toluidine blue for histologic examination and enumeration of MCs.^{E2}

Statistical analysis

All the time course and dose-response experiments were analyzed by 2-way ANOVA repeated measures in 1 factor (mixed model) followed by Bonferroni *post hoc* test. Comparison of multiple groups was analyzed by 1-way ANOVA followed by Bonferroni *post hoc* test. $P < .05$ was considered significant.

REFERENCES

- E1. Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, et al. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat Cell Biol* 2009;11:958-66.
- E2. Lilla JN, Chen CC, Mukai K, BenBarak MJ, Franco CB, Kalesnikoff J, et al. Reduced mast cell and basophil numbers and function in *Cpa3-Cre*; *Mcl-1^{fl/fl}* mice. *Blood* 2011;118:6930-8.
- E3. Gaudenzio N, Sibillano R, Marichal T, Starkl P, Reber LL, Cenac N, et al. Different activation signals induce distinct mast cell degranulation strategies. *J Clin Invest* 2016;126:3981-98.
- E4. Yano H, Wershil BK, Arizono N, Galli SJ. Substance P-induced augmentation of cutaneous vascular permeability and granulocyte infiltration in mice is mast cell dependent. *J Clin Invest* 1989;84:1276-86.

- E5. Yu M, Mukai K, Tsai M, Galli SJ. Thirdhand smoke component can exacerbate a mouse asthma model through mast cells. *J Allergy Clin Immunol* 2018;142:1618-27.e9.
- E6. Tam SY, Tsai M, Snouwaert JN, Kalesnikoff J, Scherrer D, Nakae S, et al. Rab-GEF1 is a negative regulator of mast cell activation and skin inflammation. *Nat Immunol* 2004;5:844-52.

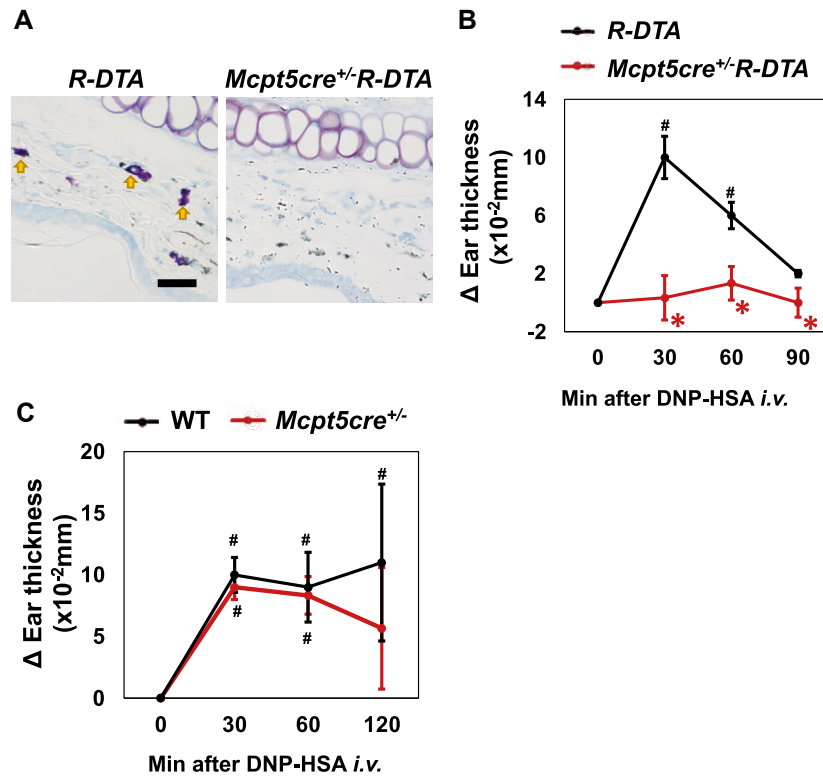


FIG E1. Skin MC depletion eliminates PCA reactions in *Mcpt5cre^{+/-} R-DTA* mice. **(A)** Ear pinna sections of *R-DTA* and *Mcpt5cre⁺ R-DTA* mice. Yellow arrows indicate MCs with toluidine blue-stained granules. **(B)** PCA reaction was abolished in *Mcpt5cre^{+/-} R-DTA* mice. **(C)** PCA reaction in wild-type (WT; *Mcpt5cre⁻*) vs *Mcpt5cre^{+/-}* mice; n = 3 mice per group. Error bars indicate SEMs. **P* < .05 for *Mcpt5cre^{+/-} R-DTA* vs *R-DTA* groups. #Δ ear thickness significantly different (*P* < .05) from 0.

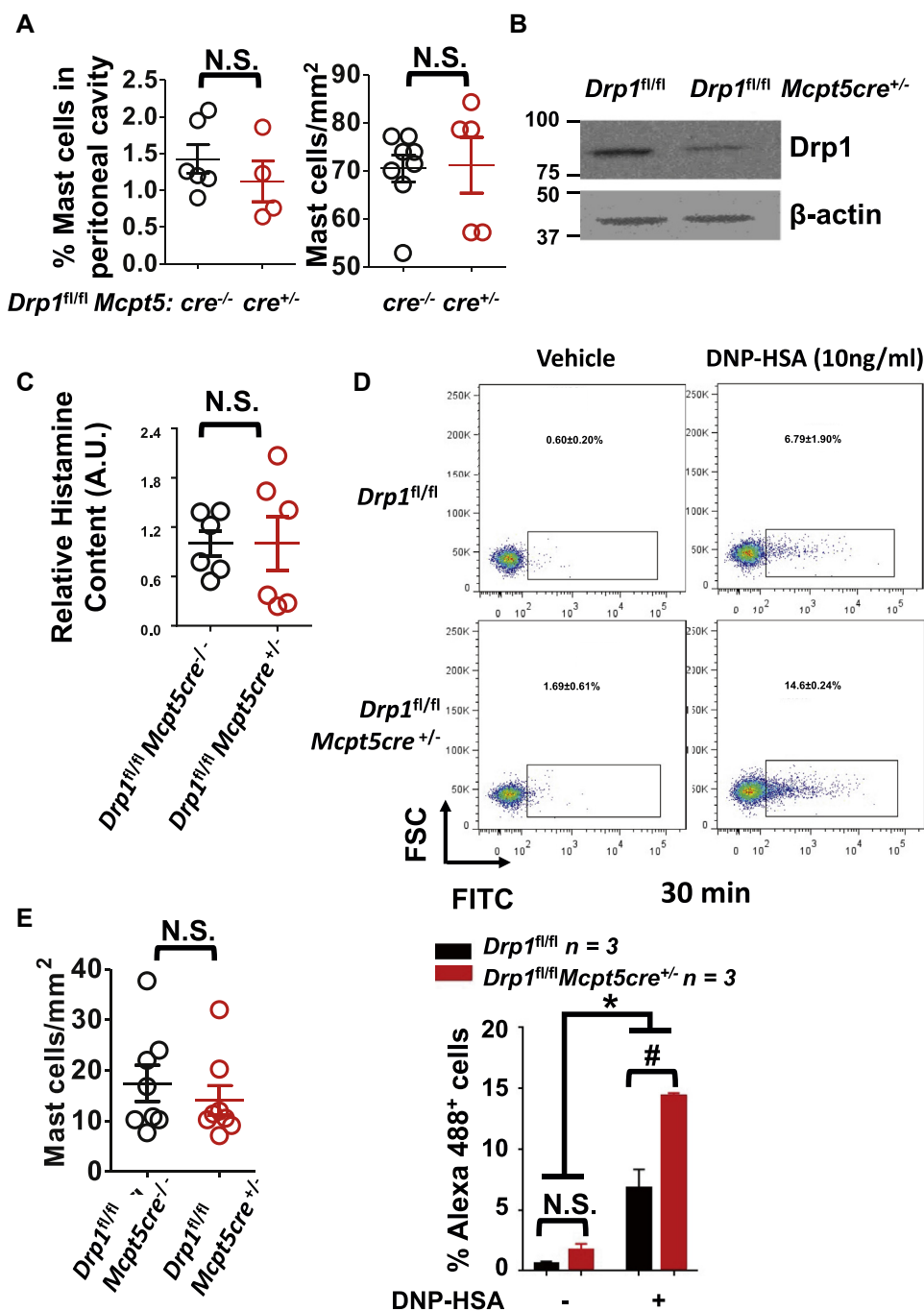


FIG E2. MC counts and histamine in *Drp1^{fl/fl} Mcpt5cre^{+/-}* and *Mcpt5cre^{+/-} → Kit^{W-sh/W-sh}* mice, Drp1 depletion, and plot of Av.A488⁺ in BMCMCs from *Drp1^{fl/fl} Mcpt5cre^{+/-}* mice. (A) FcεR1α⁺c-Kit⁺ cells expressed as percentage of all peritoneal cells; MCs/mm² ear pinnae. (B) Blots of Drp1 and β-actin (loading control). (C) Relative histamine content in BMCMCs. (D) Representative dot plots and quantification (bar graph) of Av.A488⁺ staining in cells stimulated with vehicle and 10 ng/mL DNP-HSA for 30 minutes (average ± SEM), n = 3 technical replicates, 2 mice per group. *P < .05, baseline vs 30 minutes for each mouse group; #P < .05, 30-minute results for the 2 groups. (E) Numbers of ear pinnae MCs. NS, Not significant (P > .05). Error bars indicate SEMs.

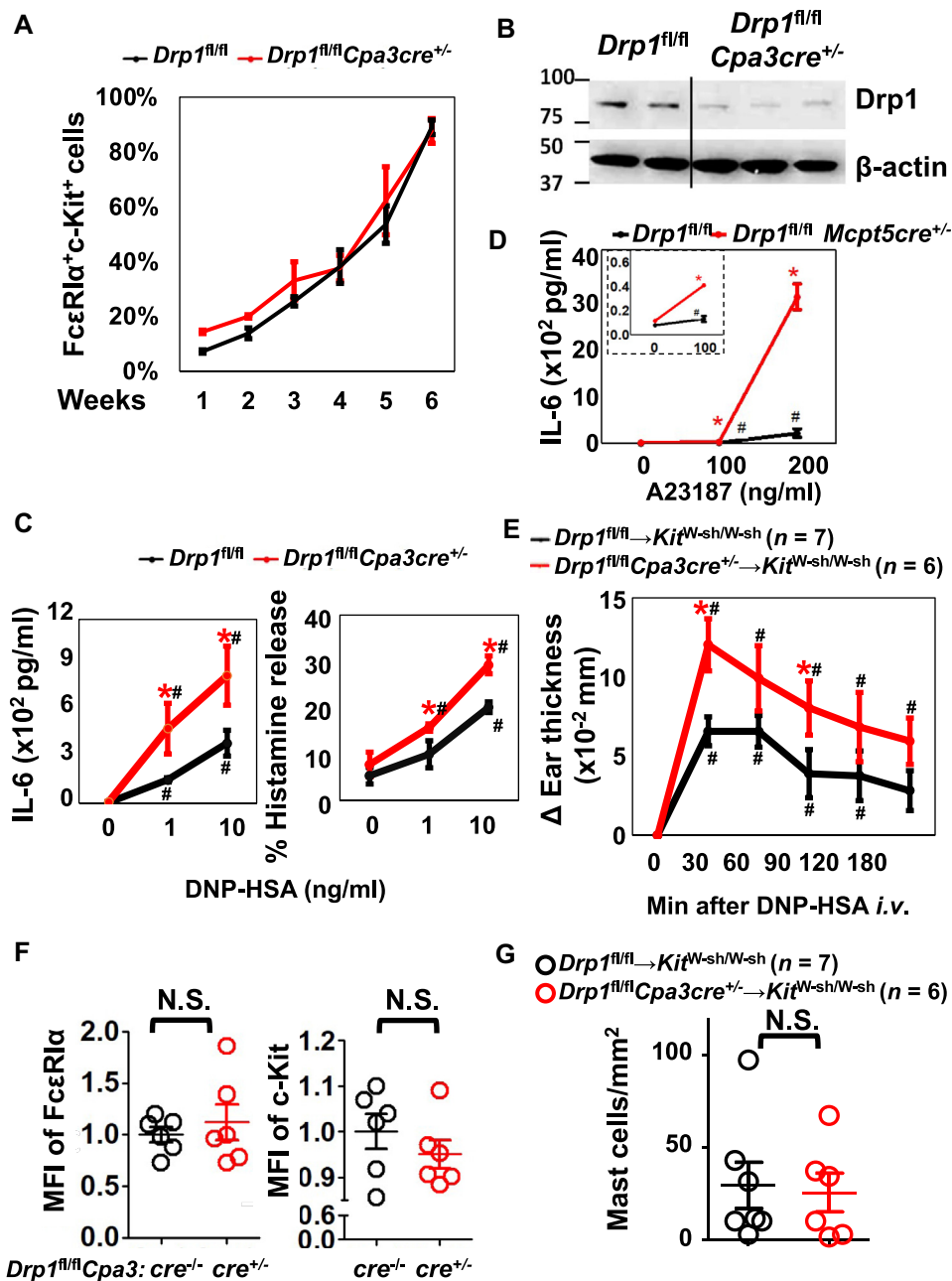


FIG E3. Enhanced FcεRI-induced activation of *Drp1^{fl/fl}Cpa3cre^{+/-}* MCs. (A) BMCMC purity. (B) Drp1 protein in BMCMC lysates (Western blot; protein ladder shown at left). (C) IL-6 release and percentage histamine release (n = 3 mice per group). (D) IL-6 release from calcium ionophore A23187-stimulated BMCMC. (E) PCA reactions (male mice). (C-E) *P < .05 between groups; #P < .05 vs zero DNP-HSA or A23187. (F) BMCMC surface FcεRIα and c-Kit (n = 3 mice per group; technical replicates). (G) Ear pinna MCs (both male and female mice). Error bars indicate SEMs. NS, Not significant.