[5]Department of Infectious Disease, Bordeaux University Hospitals, Bordeaux, France
[6]Department of Anatomopathology, Bordeaux University Hospitals, Bordeaux, France
[7]Research Unit INSERM U1035, Bordeaux, France

Correspondence
Anne-Sophie Darrigade, Dermatology Department, Saint-André Hospital, 1, rue Jean Burguet 33000 Bordeaux, France.
Email: anne-sophie.darrigade@chu-bordeaux.fr

ORCID
Anne-Sophie Darrigade https://orcid.org/0000-0003-4034-0395

REFERENCES

1. Barbaud A, Gonçalo M, Bruynzeel D, Bircher A. European Society of Contact Dermatitis. Guidelines for performing skin tests with drugs in the investigation of cutaneous adverse drug reactions. *Contact Dermatitis*. 2001;45(6):321-328.
2. Rosenblatt AE, Stein SL. Cutaneous reactions to vaccinations. *Clin Dermatol*. 2015;33(3):327-332.
3. Corbeddu M, Diociaiuti A, Vinci MR, et al. Transient cutaneous manifestations after administration of Pfizer-BioNTech COVID-19 Vaccine: an Italian single centre case series. *J Eur Acad Dermatol Venereol*. 2021. https://doi.org/10.1111/jdv.17268
4. Banerji A, Wickner PG, Saff R, et al. mRNA Vaccines to Prevent COVID-19 Disease and Reported Allergic Reactions: Current Evidence and Suggested Approach. *J Allergy Clin Immunol Pract*. 2020;S2213–2198(20):31411-31412.
5. Bianchi L, Biondi F, Hansel K, Murgia N, Tramontana M, Stingeni L. Skin tests in urticaria/angioedema and flushing to Pfizer-BioNTech SARS-CoV-2 vaccine: Limits of intradermal testing. *Allergy*. 2021;76:2605-2607. https://doi.org/10.1111/all.14839
6. Pedrosa AF, Morais P, Nogueira A, Pardal J, Azevedo F. Sweet's syndrome triggered by pneumococcal vaccination. *Cutan Ocul Toxicol*. 2013;32(3):260-261.
7. Ratzinger G, Burgdorf W, Zelger BG, Zelger B. Acute febrile neutrophilic dermatosis: a histopathologic study of 31 cases with review of literature. *Am J Dermatopathol*. 2007;29(2):125-133.

DOI: 10.1111/all.14988

Transgenic mice overexpressing the PGE_2 receptor EP_2 on mast cells exhibit a protective phenotype in a model of allergic asthma

To the Editor,

Pharmacological inhibition of airway mast cell (MC) activity has received considerable attention as a plausible anti-asthma strategy. Activation of the MC prostaglandin E_2 (PGE_2) receptor E prostanoid 2 (EP_2) may drive this potentially therapeutic mechanism. Indeed, specific EP_2 activation inhibits both murine[1] and human[2] airway MC activity *in vitro*. Furthermore, EP_2 agonism-mediated reduction in airway pathology has been linked to restricted MC activity in human[3] and in murine[4] models, and we reported upregulation of PGE_2 and of EP_2 mRNA, but not EP_1, EP_3, and EP_4, upon exposure of mice to house dust mite (HDM) aeroallergens.[5] In light of these data, we hypothesized that the EP_2-mediated improvement observed in mouse models of asthma was a consequence of reduced MC activity, and that the PGE_2-EP_2-MC axis acts as an endogenous protective mechanism.[6] To bridge this knowledge gap, we have created transgenic (TG) mice overexpressing the EP_2 receptor on MCs and have studied the response of mice sensitized to HDM aeroallergens (protocol in Supp. Figure 1). To generate the TG colony, a construct containing the MC-specific *Cma1* promoter and the EP_2 receptor coding region (*Ptger2*) was microinjected into C57BL/6OlaHsd zygotes. The TG mice were backcrossed to obtain mice overexpressing EP_2 on a BALB/c genetic background (see details in the Repository Material and Supp. Figures 2-4). All animals were bred at the Animal Facility of the Universidad Autónoma de Barcelona, and the procedures were approved by the Ethics Committee for Animal Research. To our knowledge, this is the first report of a mouse line constitutively overexpressing EP_2 selectively on MCs.

To assess the impact of MC-specific EP_2 overexpression on airway MC activity, murine chemokine mouse MC protease 1 (mMCP1) levels were measured in lung homogenates of mice sensitized to HDM aeroallergens. Non-sensitized wild-type (WT) and TG animals both had virtually no MC activity (Figure 1A), which reflected the equivalent baseline airway MC numbers (Supp. Figure 4A). mMCP1 production increased in HDM-exposed mice of both genotypes. However, mMCP1 upregulation was significantly lower in sensitized TG than in sensitized WT animals (Figure 1A). Therefore, EP_2 overexpression reduced HDM-induced MC activation *in vivo*. To confirm that this effect was specifically driven by EP_2 activation, mice were

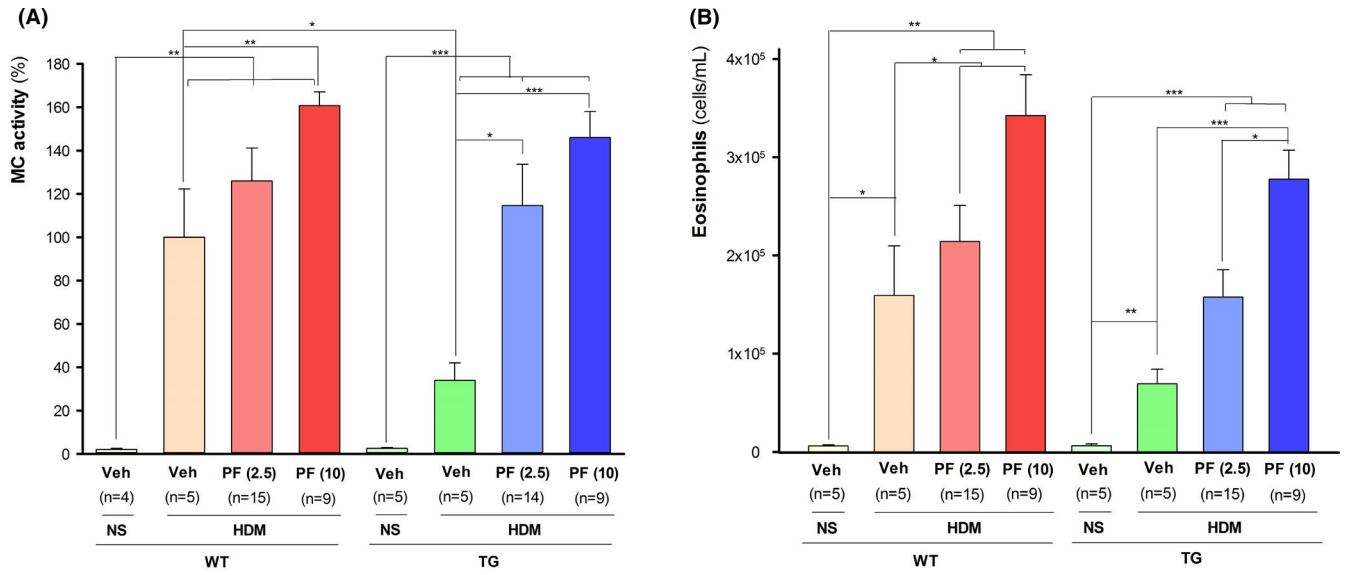

FIGURE 1 (A) HDM-induced airway MC activity in mice overexpressing EP_2. Mast cell (MC) activity was assessed by measuring mMCP1 concentration in lung homogenates normalized by total protein. WT mice exposed to HDM exhibit a significant increase in MC activity. The level of MC activation increased further in the presence of an E prostanoid receptor 2 (EP_2) antagonist (PF) at both 2.5 and 10 mg/kg. TG mice overexpressing EP_2 on MC exhibited a less pronounced increase of MC activity. Pretreatment with an EP_2 antagonist counteracted the reduced MC activity in TG mice, resulting in activity levels that were essentially equivalent between both genotypes (TG and WT). (B) HDM-induced eosinophilia in mice overexpressing EP_2. Airway inflammation was assessed by a differential cell count in bronchoalveolar lavage suspensions (see the full inflammatory cell count in Supp. Figure 5). WT mice exposed to HDM exhibited significant eosinophilia, which further increased in the presence of an EP_2 antagonist at both 2.5 and 10 mg/kg. In TG mice overexpressing EP_2 on MC, eosinophil recruitment was attenuated. After pretreatment with the EP_2 antagonist, the effect of EP_2 overexpression on eosinophilia was abrogated. Results are expressed as mean ± SEM. Statistical significance was evaluated using a two-way analysis of variance (ANOVA) with a Bonferroni post hoc test. *p<0.05, **p<0.01, and ***p<0.001. HDM (house dust mite-sensitized); MC (mast cell); NS (non-sensitized); PF (PF-0441848); TG (transgenic); Veh (vehicle); and WT (wild-type)

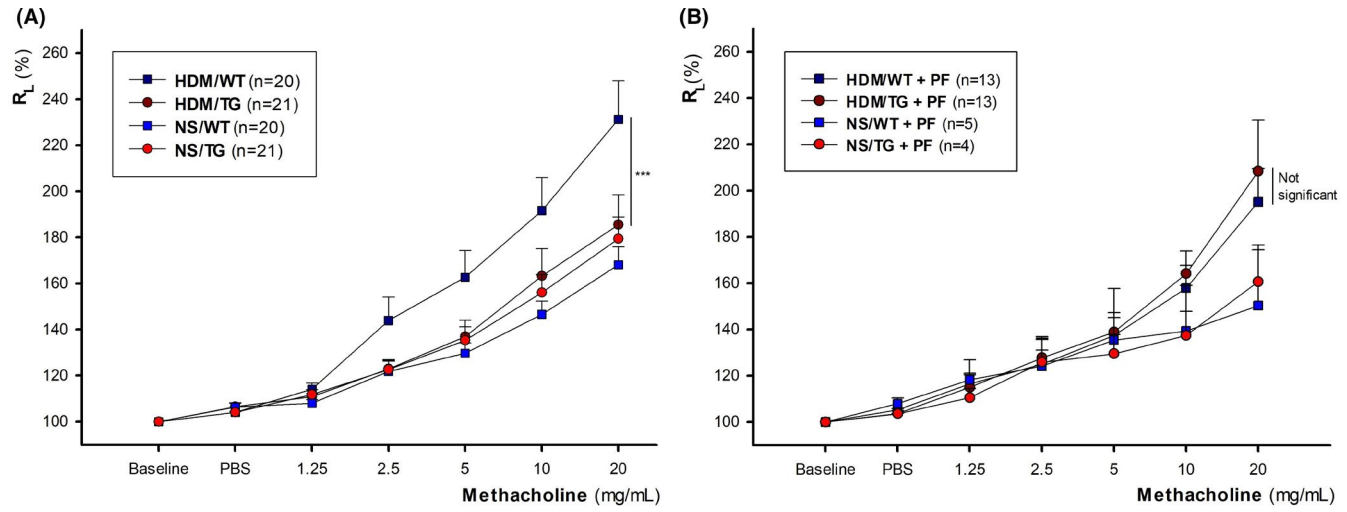

FIGURE 2 HDM-induced airway reactivity in mice overexpressing EP_2. Airway reactivity was assessed by means of invasive plethysmography and expressed as a percentage of baseline lung resistance (R_L). (A) Airway resistance in WT and TG mice exposed to HDM. Sensitization to HDM aeroallergens induced a significant increase in airway reactivity to methacholine (ie, airway hyperresponsiveness [AHR]) in WT mice (HDM/WT), while sensitized TG mice overexpressing E prostanoid receptor 2 (EP_2) (HDM/TG) did not develop AHR. (B) Airway reactivity after pretreatment with an EP_2 antagonist. In the presence of the EP_2 antagonist (PF), both sensitized WT (HDM/WT+PF) and sensitized transgenic (HDM/TG+PF) mice developed AHR. Indeed, contrary to what is observed in Figure 2A, there was no significant difference between HDM/WT and HDM/TG when the mice were pretreated with 2.5 mg/kg of PF. Results are expressed as mean ± SEM. Statistical significance was evaluated using a two-way analysis of variance (ANOVA) with a Bonferroni post hoc test. Statistically significant differences were found between baseline reactivity (NS mice) and HDM-sensitized mice. HDM (house dust mite-sensitized); R_L (lung resistance); NS (non-sensitized); PF (PF-0441848); TG (transgenic); and WT (wild-type)

pretreated with the selective EP_2 antagonist PF-04418948 prior to sensitization. In both TG and WT animals, EP_2 antagonism fostered a further increase of HDM-induced MC activity, along the lines of our recent observation in murine lung MCs *in vitro*.[1] Interestingly, the difference in HDM-induced MC activity observed between TG and WT mice was abrogated after blockade of the EP_2 receptor. All in all, this suggests that EP_2 receptor overexpression on MCs leads to an increased interation between endogenous PGE_2 and EP_2, which restricts HDM-induced MC activity.

To analyze the consequences of EP_2-driven MC inhibition, HDM-induced airway inflammation and reactivity were assessed. Inflammation was quantitated through a differential inflammatory cell count in bronchoalveolar lavage suspensions (Figure 1B). Prominent bronchovascular eosinophilic infiltration developed in mice exposed to HDM. Mimicking the MC activity pattern, the eosinophilia was lower in TG mice than in WT animals (although the difference did not achieve statistical significance). The lower eosinophilic infiltration was paralleled by a trend toward a higher number of macrophages, whereas neutrophil and lymphocyte counts remained equivalent between TG and WT mice (Supp. Figure 5). Also paralleling the effect observed on MCs, both TG and WT animals exhibited a further dose-dependent increase of inflammatory infiltrate in mice pretreated with EP_2 antagonist. However, EP_2 antagonism did not fully reverse the improvement of the inflammatory process driven by EP_2 overexpression. Analyzed in the context of previous data where reduced bronchovascular inflammation was demonstrated in HDM-sensitized mice treated with a selective EP_2 agonist,[4] the current data support the view that an endogenous PGE_2-EP_2-MC compensatory mechanism may attenuate HDM-driven eosinophilic inflammation. Finally, invasive plethysmography was used to measure airway reactivity to methacholine (Figure 2). Non-sensitized TG and WT animals exhibited no difference in baseline airway constriction upon increasing doses of methacholine (Figure 2A). HDM-sensitized WT animals developed the expected level of airway hyperresponsiveness (AHR) after a 10-day exposure to HDM aeroallergens. Indeed, as per ANOVA, lung resistance (R_L) was significantly higher in sensitized than in non-sensitized WT mice. By contrast, the methacholine dose-response curve of sensitized TG animals overexpressing EP_2 on MCs showed no induction of AHR upon exposure to HDM. Indeed, contrary to WT animals, the R_L of sensitized and non-sensitized TG mice did not differ. This finding agrees with our previous observations after exogenous EP_2 activation.[4] Pretreatment of mice with the EP_2 antagonist PF-0418948 (Figure 2B) abrogated the beneficial effect exerted by EP_2 overexpression on HDM-induced airway reactivity. Contrary to mice that were not pretreated with PF-0418948, both TG and WT animals exhibited similar levels of AHR after methacholine treatment in the presence of 2.5 mg/kg of the antagonist.

We have shown that MC-specific EP_2 overexpression protects from HDM aeroallergen-induced airway pathology in a IgE-dependent murine asthma model. A direct connection between reduced MC activity and improvement of airway inflammation and reactivity has been demonstrated. The finding that EP_2 antagonism worsens HDM-induced harmful effects in WT animals, and reverses the benefit exerted by EP_2 overexpression, supports the hypothesis that the PGE_2–EP_2–MC axis acts as a natural defensive barrier. This is consistent with the recent observation that PGE_2 protects from the development of anaphylaxis and MC hyperresponsiveness.[7] It also builds on our hypothesis of compensatory PGE_2 and EP_2 upregulation in mice exposed to HDM[5] and on the preventive effect exerted by exogenous EP_2 activation.[4] In a recent consensus report,[8] the European Academy of Allergy and Clinical Immunology (EAACI) Task Force discussed the need to further research the role of eicosanoids in asthma and allergic diseases. The authors specifically mention the bronchoprotective and anti-inflammatory properties of PGE_2 in the lungs, remind us of its ability to reduce MC activity, and request further studies with selective $EP_{1\text{-}4}$ analogues. By using an alternative approach, we hope to have contributed to the link between eicosanoid biology and asthma pathophysiology. Further characterization of the cellular and molecular events involved in the PGE_2–EP_2–MC axis in the newly created C.B6-Tg (*Cma1-Ptger2*) mice will undoubtedly unravel new anti-asthma therapeutic target candidates and offer future research opportunities.

FUNDING INFORMATION

Instituto de Salud Carlos III, Grant/Award Number: PI18/01702

ACKNOWLEDGMENTS

This study was supported by a grant from Instituto de Salud Carlos III of the Spanish Ministry of Science, Innovation and Universities (Ref. PI18/01702).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Urbano A involved in induction of mice's sensitization and conduction of airway hyperreactivity and inflammation studies, as well as mast cell activity assessment; data analysis and interpretation; and manuscript writing. Plaza J contributed to early stage airway hyperreactivity and inflammation studies in HDM-sensitized mice, as well as mast cell activity assessment. Turon S contributed to transgenic mice colony generation and handling. Pujol A contributed to transgenic mice colony generation and handling; manuscript revision. Costa-Farré C contributed to mice handling for airway hyperreactivity studies. Marco A involved in assessment of airway inflammation and interpretation. Picado C involved in study design and data interpretation, and manuscript revision. Torres R involved in experiment supervision and support in transgenic mice colony generation; data analysis and interpretation; and manuscript revision. de Mora F involved in study design, experiment supervision, and data analysis and interpretation; manuscript writing.

Adrián Urbano[1]
Judith Plaza[1]
Sandra Turon[2]

Anna Pujol[2]
Cristina Costa-Farré[3]
Alberto Marco[3]
César Picado[4,5]
Rosa Torres[1]
Fernando de Mora[1]

[1]*Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona, Barcelona, Spain*
[2]*Transgenic Animal Unit, Center of Animal Biotechnology and Gene Therapy, Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain*
[3]*Department of Surgery and Animals Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain*
[4]*Department of Pneumology and Respiratory Allergy, Hospital Clínic, IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer), Universitat de Barcelona, Barcelona, Spain*
[5]*CIBERES (Centro de Investigación Biomédica en Red de Enfermedades Respiratorias), Barcelona, Spain*

Correspondence
Fernando de Mora, Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona, Barcelona, Spain.
Email: fernando.demora@uab.cat

Rosa Torres and Fernando de Mora have contributed equally to this study.

ORCID

Adrián Urbano https://orcid.org/0000-0002-4107-1644
Judith Plaza https://orcid.org/0000-0003-2217-3095
Sandra Turon https://orcid.org/0000-0001-9526-8584
Anna Pujol https://orcid.org/0000-0002-3484-598X
Cristina Costa-Farré https://orcid.org/0000-0002-0224-6747
Alberto Marco https://orcid.org/0000-0003-1680-0822
César Picado https://orcid.org/0000-0001-7400-4993
Rosa Torres https://orcid.org/0000-0002-9109-4035
Fernando de Mora https://orcid.org/0000-0002-3002-6004

REFERENCES

1. Plaza J, Torres R, Urbano A, Picado C, de Mora F. In vitro and in vivo validation of EP2-receptor agonism to selectively achieve inhibition of mast cell activity. *Allergy Asthma Immunol Res*. 2020;12:712. https://doi.org/10.4168/aair.2020.12.4.712
2. Kay LJ, Yeo WW, Peachell PT. Prostaglandin E 2 activates EP 2 receptors to inhibit human lung mast cell degranulation. *Br J Pharmacol*. 2006;147:707-713. https://doi.org/10.1038/sj.bjp.0706664
3. Säfholm J, Manson ML, Bood J, et al. Prostaglandin E 2 inhibits mast cell – dependent bronchoconstriction in human small airways through the E prostanoid subtype 2 receptor. *J Allergy Clin Immunol*. 2015;136:1232-1240. https://doi.org/10.1016/j.jaci.2015.04.002
4. Serra-Pages M, Torres R, Plaza J, et al. Activation of the Prostaglandin E2 receptor EP2 prevents house dust mite-induced airway hyperresponsiveness and inflammation by restraining mast cells' activity. *Clin Exp Allergy*. 2015;45:1590-1600. https://doi.org/10.1111/cea.12542
5. Herrerias A, Torres R, Serra M, et al. Activity of the cyclooxygenase 2-prostaglandin-E prostanoid receptor pathway in mice exposed to house dust mite aeroallergens, and impact of exogenous prostaglandin E2. *J Inflamm*. 2009;6:1-9. https://doi.org/10.1186/1476-9255-6-30
6. Torres R, Picado C, de Mora F. The PGE2-EP2-mast cell axis: an antiasthma mechanism. *Mol Immunol*. 2015;63:61-68. https://doi.org/10.1016/j.molimm.2014.03.007
7. Rastogi S, Willmes DM, Nassiri M, Babina M, Worm M. PGE2 deficiency predisposes to anaphylaxis by causing mast cell hyper-responsiveness. *J Allergy Clin Immunol*. 2020. https://doi.org/10.1016/j.jaci.2020.03.046
8. Sokolowska M, Enrico RG, Diamant Z, et al. Current perspective on eicosanoids in asthma and allergic diseases: EAACI Task Force consensus report, part I. *Allergy Eur J Allergy Clin Immunol*. 2021;76:114-130. https://doi.org/10.1111/all.14295

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

DOI: 10.1111/all.14997

Type 2 biomarker expression (FeNO and blood eosinophils) is higher in severe adult-onset than in severe early-onset asthma

To the Editor,

Severe asthma is predominantly associated with a type 2 inflammatory pattern[1]; however, it is unclear whether the expression of type 2 biomarkers differs between severe early-onset asthma (SEA) and severe adult-onset asthma (SAA). This issue is of particular importance for the question whether the age of asthma onset can be a

Abbreviations: FeNO, fractional exhaled nitric oxide; GINA, global initiative for asthma; IgE, immunoglobulin E; OCS, oral corticosteroid; SAA, severe adult-onset asthma; SEA, severe early-onset asthma; UK, United Kingdom; UKSAR, United Kingdom severe asthma registry.