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SUPPORTING INFORMATION

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DOI: 10.1111/all.15596

Depletion of Mcpt8-expressing cells reduces lung mast cells in mice with experimental asthma

To the Editor,

Genetically engineered mouse models have exploited the basophil marker mast cell protease-8 (mMCP-8) to dissect the role of basophils in inflammatory diseases.[1–3] However, mast cell progenitors (MCps) also express *Mcpt8* transcripts in the allergic mouse lung.[4] Here, we investigated whether depletion of *Mcpt8*-expressing cells impacts

P. Abigail Alvarado-Vazquez and Eduardo I. Cardenas equal contribution.

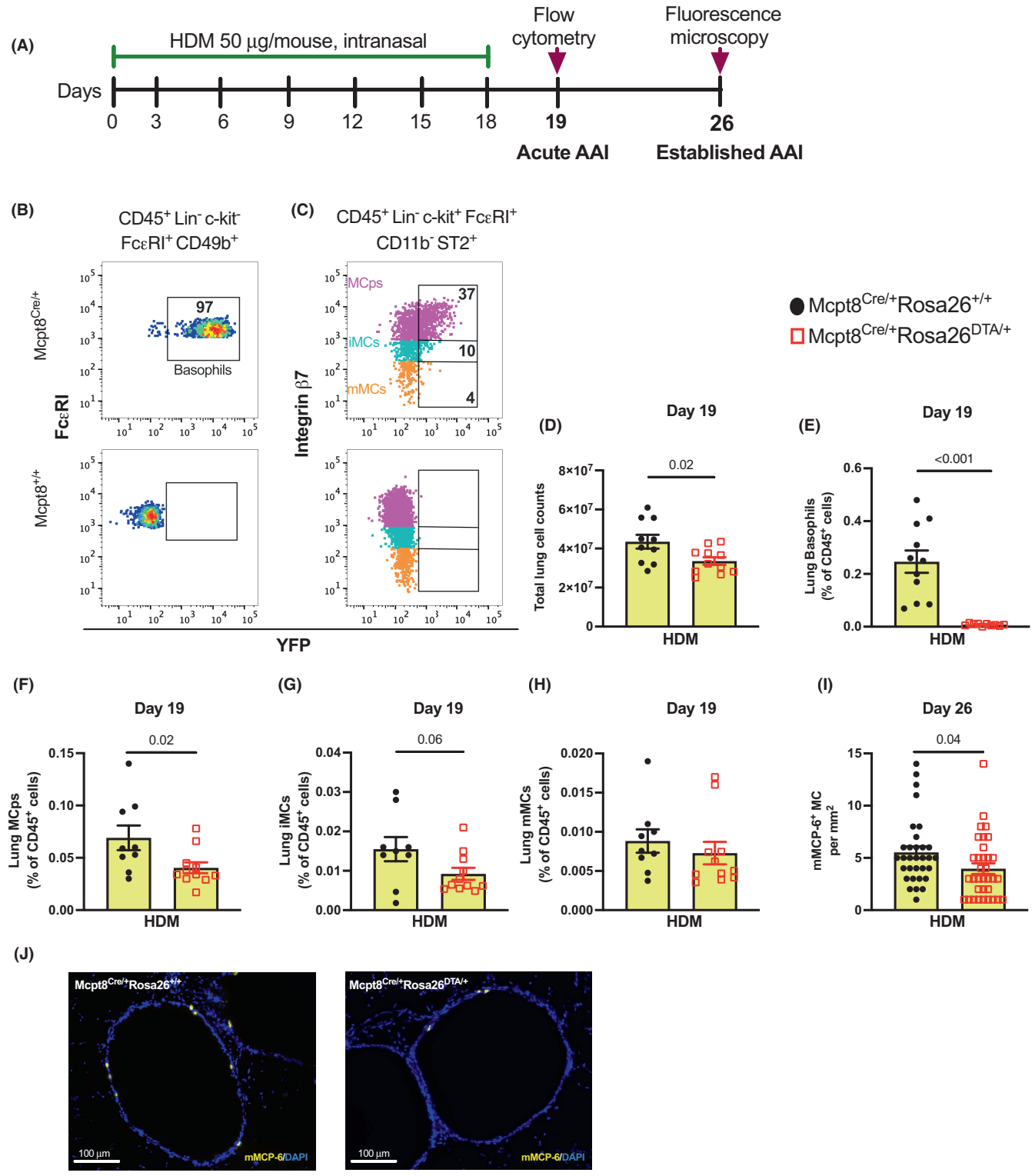

FIGURE 1 Basophil-deficient Mcpt8$^{Cre/+}$Rosa26$^{DTA/+}$ mice with HDM-AAI have decreased lung MCps, which later translates into decreased mMCP-6^{+} MCs. (A) Protocol for inducing AAI. (B) Representative YFP signal in lung basophils, (C) MCps, iMCs, and mMCs in a Mcpt8$^{Cre/+}$ (upper panel) or Mcpt8$^{+/+}$ mouse (lower panel) with acute AAI (d19). (D–H) Total lung cells, and the frequency of lung basophils, MCps, iMCs, mMCs quantified in basophil-deficient Mcpt8$^{Cre/+}$Rosa26$^{DTA/+}$ and Mcpt8$^{Cre/+}$Rosa26$^{+/+}$ mice with acute AAI. (I, J) Lung mMCP6^{+} cells were quantified in established AAI (day 26). Each point represents one picture from three mice per group. (D–H) Each point represents one mouse from three independent experiments. Bars represent the mean ± SEM. *$p < .05$ using Student's *t*-test

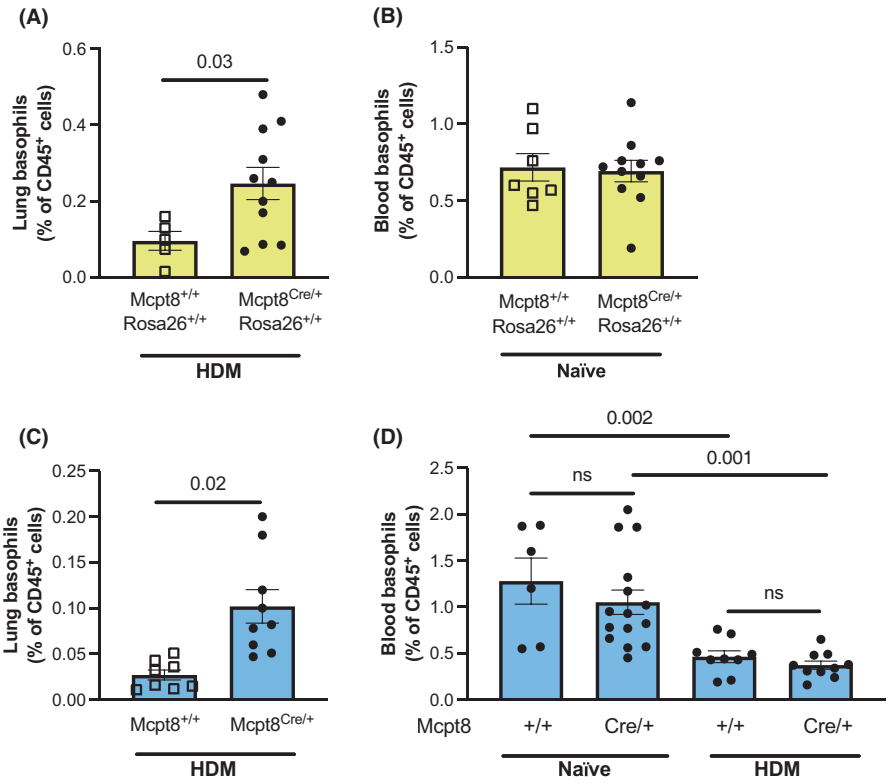

FIGURE 2 Mcpt8$^{Cre/+}$ mice show increased lung basophils during acute HDM-AAI. (A) Lung and (B) blood basophil frequency in Mcpt8$^{Cre/+}$Rosa26$^{+/+}$ and Mcpt8$^{Cre/+}$Rosa26$^{+/+}$ mice with acute AAI. (C, D) Lung and blood basophil frequency in Mcpt8$^{Cre/+}$ and Mcpt8$^{+/+}$ mice with acute AAI (C, D) or naive mice (D). Each point represents one mouse. Bars represent the mean ± SEM of three independent experiments. *$p < .05$ using Student's *t*-test

lung MC populations in a mouse model of house dust mite-induced allergic airway inflammation (AAI) (Figure 1A; Methods in Appendix S1). In this model, MCps are recruited to the lung in acute AAI and later expand the MC population.[5]

The Mcpt8$^{Cre/+}$ strain expresses a yellow fluorescent protein (YFP) in an *Mcpt8*-dependent manner.[3] As naive mice have extremely few MCps and mature MCs (mMCs), we were unable to reliably quantify the YFP-signal in these cells. Our flow cytometry gating strategy (Figure S1),[5] which identifies basophils and three lung MC populations in acute AAI: MCps, induced MCs (iMCs), and mMCs, was used to investigate mMCP-8 expression. As expected, 98 ± 0.5% of lung basophils from Mcpt8$^{Cre/+}$ mice with acute AAI were YFP$^+$ (Figure 1B). However, 37 ± 6% of MCps and 10 ± 2% of iMCs had YFP signal, while only 4 ± 1% of mMCs were YFP$^+$ (Figure 1C). In agreement, peritoneal mMCs from Mcpt8$^{Cre/+}$ mice showed no YFP-signal in a sepsis model.[2] We were unable to detect YFP signal in bronchoalveolar lavage (BAL) eosinophils, neutrophils, alveolar macrophages, and T cells from mice with acute AAI (not shown). Hence, mMCP-8 is primarily expressed by lung basophils and MCps in acute AAI.

Next, basophil-deficient mice were generated by crossing Mcpt8$^{Cre/+}$ and Rosa26$^{DTA/+}$ mice. The Rosa26$^{DTA/+}$ strain carries the diphtheria toxin A (DTA) gene in the Rosa26 locus downstream of a loxP-flanked stop cassette.[6] Thus, *Mcpt8*-dependent Cre-expression leads to DTA-mediated cell ablation. In acute AAI, Mcpt8$^{Cre/+}$Rosa26$^{DTA/+}$ mice had fewer total lung cells and were deficient (~96% reduction) in lung basophils (Figure 1D,E). Quantification of lung MC populations revealed a 41% decrease in lung MCps in Mcpt8$^{Cre/+}$Rosa26$^{DTA/+}$ mice (Figure 1F). However, iMCs and mMCs were not significantly altered in acute AAI (Figure 1G,H), which was expected as resident mMCs and iMCs exhibited low mMCP-8 expression during acute AAI. This is in agreement with our previous study,[5] showing that the mMC population is not yet expanded in mice with acute AAI. Moreover, a previous publication reported no changes in peritoneal mMCs following short-term DTA-driven depletion of *Mcpt8*-expressing cells in vivo.[1] In our study, the absence of basophils and partial loss of MCps did not affect total BAL cells, eosinophils, neutrophils, alveolar macrophages, and CD8$^+$ T cells but resulted in an increased frequency of CD4$^+$ T cells (Figure S2A–F).

To test whether the *Mcpt8*-mediated reduction in lung MCps would later impact the number of AAI-induced mMCs, lung sections were analyzed for tryptase (mMCP-6) positive MCs on Day 26 when AAI is established in the lung. Mcpt8$^{Cre/+}$Rosa26$^{DTA/+}$ mice had in average a 28% reduction in mMCP-6$^+$ lung cells during established-AAI (Figure 1I,J). Thus, *Mcpt8*-driven deletion of lung MCps translates into a reduction in total lung MCs at a later time point.

While investigating the effect of *Mcpt8*-mediated cell deletion, we unexpectedly found that Mcpt8$^{Cre/+}$Rosa26$^{+/+}$ mice with acute AAI had ~threefold increase in lung basophils compared to Mcpt8$^{+/+}$Rosa26$^{+/+}$ (wildtype) littermates (Figure 2A). Nevertheless, the levels of blood circulating basophils in naive mice were unaltered (Figure 2B). The increase in lung basophils was not due to crossing with Rosa26$^{DTA/+}$ mice, because Mcpt8$^{Cre/+}$ mice with acute AAI also had a higher frequency of lung basophils than wildtype littermates (Figure 2C). Still, the blood basophil frequency was similar between Mcpt8$^{Cre/+}$ mice and their wildtype controls both in naive and in those with acute AAI (Figure 2D). Moreover, lung MC populations were also comparable between Mcpt8$^{Cre/+}$ and Mcpt8$^{+/+}$ mice with

acute AAI (Figure S2G–I). We speculate that the YFP-, IRES-, and Cre-recombinase-containing insert[3] in Mcpt8$^{Cre/+}$ mice enhances basophil development or survival in the AAI lung.

In conclusion, a fraction of lung MCps expresses *Mcpt8* during acute HDM-AAI, causing mice with *Mcpt8*-driven cell ablation to have reduced lung MCps, and later reduced mMCs. Mcpt8$^{Cre/+}$ mice with acute HDM-AAI have an increased frequency of lung basophils, which might exacerbate the role of basophils in inflammatory models. Therefore, we suggest caution when interpreting the findings from *Mcpt8*-dependent models.

ACKNOWLEDGMENTS

We thank professor Axel Roers and professor David Voehringer for kindly providing the Rosa26-DTA mice. Flow cytometry was performed in the BioVis Facility at the Science for Life Laboratory, Uppsala, Sweden.

FUNDING INFORMATION

This work was supported by grants to JH from the Swedish Research Council, the Swedish Heart-Lung Foundation, Knut and Alice Wallenberg Foundation, Hans von Kantzow Foundation, Ruth and Nils-Erik Stenbäck Foundation; and to EIC from the Gustaf Adolf Johansson Foundation, Agnes and Mac Rudberg Foundation, and P. O. Zetterling Foundation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

DOI: 10.1111/all.15619

Reduced intra-subject variability of an automated skin prick test device compared to a manual test

To the Editor,

Respiratory allergies affect 30%–40% of individuals worldwide and represent a major health-economic problem.[1] Identification of the triggering or causative allergens in symptomatic patients is based on skin prick test or serum-specific IgE analysis in addition to a detailed medical history by the physician.[2,3] Skin prick test (SPT)