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Letter

Mast cells partly contribute to allergic enteritis development: findings in two different mast cell-deficient mice

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To the editor

Allergic enteritis (AE) is a gastrointestinal form of food allergy (1). Compared to other clinical phenotypes of allergy, the pathomechanisms of AE have not been elucidated. In this study, we provide evidence, based on studies of two mast cell-deficient mouse strains ($KIT^{(W\text{-}sh/W\text{-}sh)}$ bearing the W-sash (W(sh)) inversion mutation and $Cpa3^{Cre/+}$ lacking mast cells due to Cre-mediated mast cell eradication) (2) that mast cells contribute to the development of AE, leading to eosinophil, but not neutrophil, accumulation by inducing CC chemokine ligand (CCL) 1 in the inflamed intestines.

Sensitization with ovalbumin (OVA, an egg white allergen) plus alum and challenge with an egg white (EW) diet (immunization schedule in Fig. S1A) induced clinical signs (body temperature and weight reduction) (Fig. S1B-C) and inflammatory features (e.g., villous atrophy, edema, granulocyte accumulation and mast cell activation) of AE in wild type (WT) and $Cpa3^{+/+}$ (litter mate) mice (Fig. 1, Fig. S1D-E).

Compared to WT mice, each mast cell-deficient strain had significantly reduced development of clinical signs (Fig. S1B-C) but exhibited AE differently. By histology, we found that inflammation in AE was significantly reduced in $KIT^{W\text{-}sh/W\text{-}sh}$ mice (Fig. 1A) but only

partly in $Cpa3^{Cre/+}$ mice (Fig. 1B). FACS analysis showed that $KIT^{W\text{-}sh/W\text{-}sh}$ mice had reduced numbers of both eosinophils ($CD45^+CD11b^+SiglecF^+$cells) and neutrophils ($CD45^+CD11b^+SiglecF^-Ly6G^+$cells), whereas $Cpa3^{Cre/+}$ mice had reduced eosinophils, but not neutrophils, in intestinal tissues (Fig. 2A, Fig. S2A-B).

The W-sh mutation broadly affects c-Kit expression in myeloid precursor cells and increases granulocytic myeloid-derived suppressor cells (2), which may contribute to reduced neutrophil accumulation and inflammation in $KIT^{W-sh/W-sh}$ mice. Importantly, both mast cells and eosinophils are observed in biopsy specimens upon microscopic inspection in most patients with AE (1). Yagi *et al.* showed that the presence of neutrophils in intestinal biopsies is an indicator of a severe course of AE (3). These studies, including ours, suggest that targeting both eosinophils and neutrophils may be necessary to suppress the development of AE.

Among detectable eosinophil chemoattractants, both mast cell-deficient mice reduced CCL1, but not IL-5 or CCL8 expression in intestinal tissues, whereas significant reduction of CCL11 was observed only in $KIT^{W\text{-}sh/W\text{-}sh}$ mice (Fig. 2B-D, Fig. S3B).The results suggest that mast cells lead to eosinophil accumulation by inducing CCL1 expression in the AE tissues. This is consistent with our previous study showing that deficiency of CC chemokine receptor (CCR) 8, the receptor of CCL1, reduced eosinophil accumulation and the development of AE, but only partly (5). Gonzalo *et al.* showed that an axis of mast cell-derived CCL1 and CCR8 contributes to the development of Th2-mediated lung inflammation in a mouse model (5). However, our immunohistochemistry showed that mast cells (toluidine blue positive cells) and monocytes/macrophage populations ($CD68^+$ cells) are not the main CCL1 producer (Fig. S3). It appears unlikely that T-cell population produces CCL1, because the levels of Th2-, or Treg-associated cytokines in intestinal homogenates, the frequency or response of $CD4^+$ T-cells and Treg cells in mesenteric lymph nodes, and the levels of OVA-specific IgE were comparable between mast cell-deficient mice and their controls (Figs. S4, S5). Knipfer *et al.* showed that CCL1 is produced by innate lymphoid cells (ILC)2, supporting their capacity to protect against helminthic infections in the intestines (6). Further study is necessary to identify whether ILC2 represent the origin of CCL1 in AE tissues.

To our knowledge, the present study is the first to provide consistent results, using two independent mast cell-deficient mouse strains, regarding the role of mast cells and expression of CCL1 in the eosinophil recruitment at sites of AE. This study thus offers implications for establishing AE treatments that target infiltrating leucocytes in AE. (Word count 598 words/600 words)

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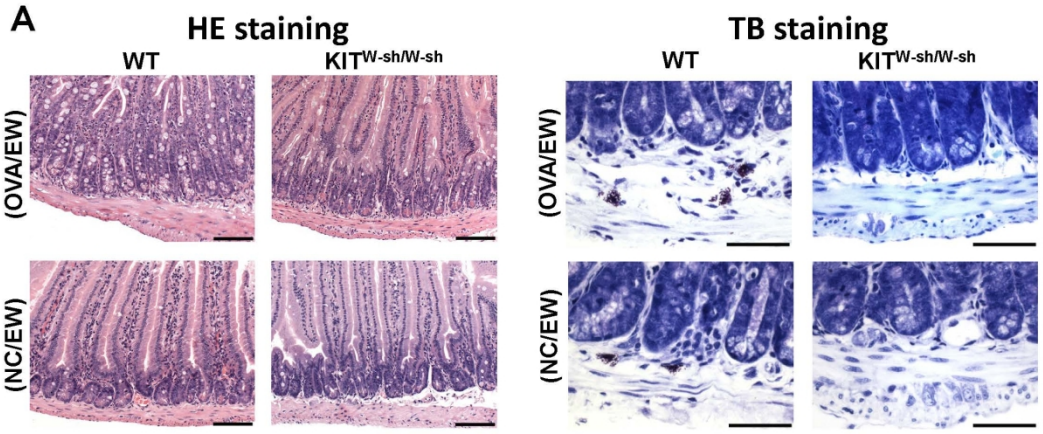
Figure legends
Fig. 1: Reduced development of AE in mast cell-deficient mice. (A) WT and $KIT^{W\text{-}sh/W\text{-}sh}$ and (B) $Cpa3^{+/+}$ and $Cpa3^{Cre/+}$ mice were i.p. sensitized with OVA plus ALUM, or treated with

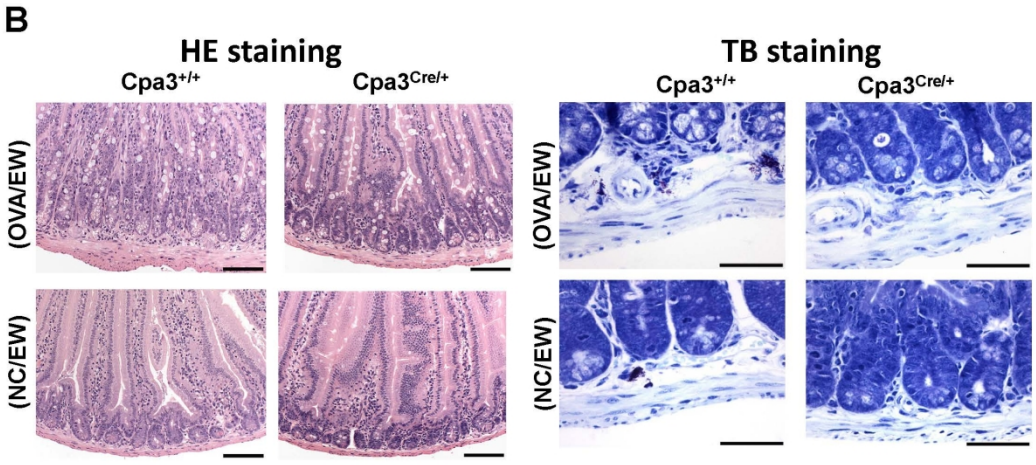
PBS, and fed EW-diet for 7 days. The jejunum stained with H&E (left, 200x magnification, scale bar 100 µm) or, to stain mast cells, toluidine blue (right, 630x magnification, scale bar 50 µm). OVA/EW: OVA-sensitized and EW diet-fed mice, NC/EW: non-sensitized and EW diet-fed mice. Statistics: (1) *vs* WT (NC/EW); (2) *vs* $KIT^{W\text{-}sh/W\text{-}sh}$ (OVA/EW); (3) *vs* $KIT^{W\text{-}sh/W\text{-}sh}$ (NC/EW); (4) vs $Cpa3^{+/+}$ (NC/EW); (5) vs $Cpa3^{Cre/+}$ (NC/EW). * *p<0.05., **p<0.01.*

Fig. 2: Reduced eosinophils in mast cell-deficient mice. WT and $KIT^{W\text{-}sh/W\text{-}sh}$ and $Cpa3^{+/+}$ and $Cpa3^{Cre/+}$ mice (n=4/group) were sensitized with OVA plus ALUM and fed EW-diet. (A) The frequencies of $CD45^{+}CD11b^{+}$ cells in jejunum lamina propria cells, and eosinophils ($SiglecF^{+}CD11b^{+}$ cells) and neutrophils ($Ly6G^{+}CD11b^{+}$ cells) in the $CD45^{+}CD11b^{+}$ cells, were determined by FACS. The average of three independent experiments is shown. The concentrations of (B) CCL1, (C) CCL8, and (D) CCL11 in intestinal homogenates were measured by ELISA. Data are pooled of three independent experiments. OVA/EW: OVA-sensitized and EW diet-fed mice, NC/EW: non-sensitized and EW diet-fed mice. * *p<0.05., **p<0.01.*



Inflammation levels in WT and KIT$^{W\text{-}sh/W\text{-}sh}$ mice

Group	Inflammation	Villi	Edema	Total
WT (OVA/EW)	2.50**(1) ± 1.73	0.75 ± 0.5	0.75 ± 0.5	4.00**(1), *(2) ± 2.2
WT (NC/EW)	0.20 ± 0.45	0.20 ± 0.5	0.20 ± 0.5	0.60 ± 0.9
KIT$^{W\text{-}sh/W\text{-}sh}$ (OVA/EW)	1.25*(3) ± 1.3	0.38 ± 0.5	0.75 ± 0.5	2.25*(3) ± 1.0
KIT$^{W\text{-}sh/W\text{-}sh}$ (NC/EW)	0.00 ± 0.0	0.50 ± 0.6	0.00 ± 0.0	0.50 ± 0.6



Inflammation levels in Cpa3$^{+/+}$ and Cpa3$^{Cre/+}$ mice

Group	Inflammation	Villi	Edema	Total
Cpa3$^{+/+}$ (OVA/EW)	2.25**(4) ± 1.7	0.25 ± 0.5	0.50 ± 0.6	3.00**(4) ± 2.5
Cpa3$^{+/+}$ (NC/EW)	0.00 ± 0.0	0.25 ± 0.5	0.25 ± 0.5	0.50 ± 0.9
Cpa3Cre/+ (OVA/EW)	1.90**(5) ± 1.3	0.20 ± 0.4	0.60 ± 0.5	2.70**(5) ± 1.8
Cpa3Cre/+ (NC/EW)	0.00 ± 0.0	0.17 ± 0.4	0.00 ± 0.0	0.17 ± 0.4

Figure 1_Blanco-Pérez et al.

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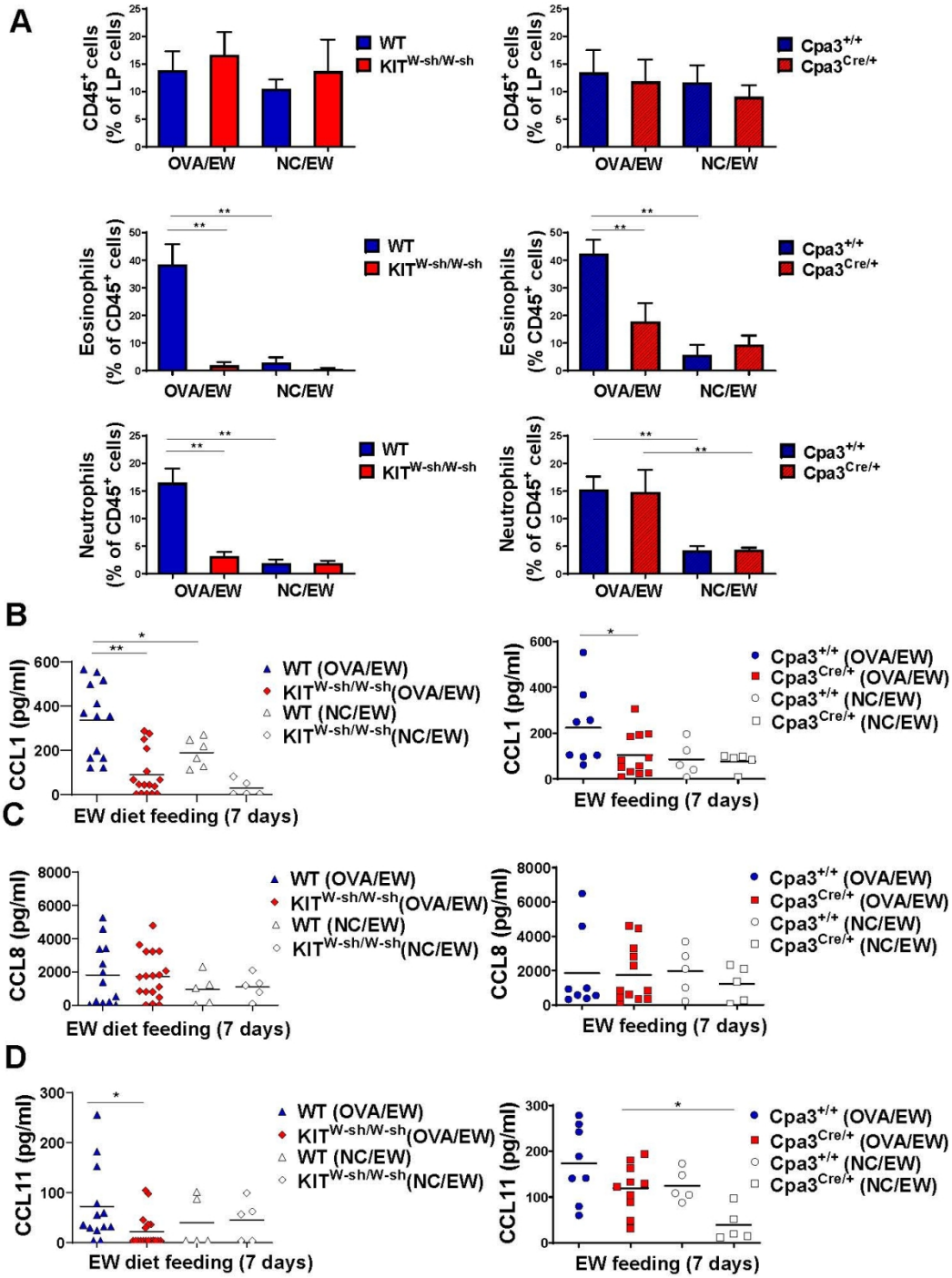

Figure 2_Blanco-Pérez et al.

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