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

To the editor,

Allergic enteritis (AE) is a gastrointestinal form of food allergy. Compared with other clinical phenotypes of allergy, the pathomechanisms of AE have not been elucidated. In this study, we provide evidence, based on the studies of two mast cell-deficient mouse strains (KIT^{W-sh/W-sh} bearing the W-sash (W(sh)) inversion mutation and Cpa3^{Cre/+} lacking mast cells due to Cre-mediated mast cell eradication)\(^\text{1}\) that mast cells contribute to the development of AE.

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**FIGURE 1** Reduced development of AE in mast cell-deficient mice. (A) WT and KIT^{W-sh/W-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, 200× magnification, scale bar 100 µm) or, to stain mast cells, toluidine blue (right, 630× 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-sh/W-sh} (OVA/EW); (3) vs KIT^{W-sh/W-sh} (NC/EW); (4) vs Cpa3^{+/+} (NC/EW); and (5) vs Cpa3^{Cre/+} (NC/EW). *p < .05, **p < .01.
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 Figure S1A) induced clinical signs (body temperature and weight reduction) (Figure S1B-C) and inflammatory features (eg, villous atrophy, edema, granulocyte accumulation, and mast cell activation) of AE in wild type (WT) and Cpa3+/+ (litter mate) mice (Figures 1 and S1D-E).

Compared with WT mice, each mast cell-deficient strain had significantly reduced development of clinical signs (Figure S1B-C) but exhibited AE differently. By histology, we found that inflammation in AE was significantly reduced in KIT W−sh/W−sh mice (Figure 1A) but only partly in Cpa3Cre/+ mice (Figure 1B). FACS analysis showed that KIT W−sh/W−sh mice had reduced numbers of both eosinophils (CD45+CD11b+SiglecF+ cells) and neutrophils (CD45+CD11b+SiglecFLy6G+ cells), whereas Cpa3Cre/+ mice had reduced eosinophils, but not neutrophils, in intestinal tissues (Figures 2A and S2A-B).

The W-sh mutation broadly affects c-Kit expression in myeloid precursor cells and increases granulocytic myeloid-derived suppressor cells, 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 al3 showed that the presence of neutrophils in intestinal biopsies is an indicator of a severe course of AE. These studies, including ours, suggest that targeting both eosinophils and neutrophils may be necessary to suppress the development of AE.

FIGURE 2 Reduced eosinophils in mast cell-deficient mice. WT and KIT W−sh/W−sh and Cpa3+/+ and Cpa3Cre/+ 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 < .05, **p < .01
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. 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. However, our immunohistochemistry showed that mast cells (toluidine blue positive cells) and monocytes/macrophage populations (CD68+ cells) are not the main CCL1 producer (Figure 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 (Figures S4 and S5). Knipfer et al. showed that CCL1 is produced by innate lymphoid cells (ILC)2, supporting our controls (Figures S4 and 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. 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 leukocytes in AE.

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CONFLICT OF INTEREST
Statement of COI is in Appendix S1

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REFERENCES

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