EoE Biopsies have Elevated and Activated Mast Cells that Produce Cytokines and Chemokines that Drive Disease Pathogenesis

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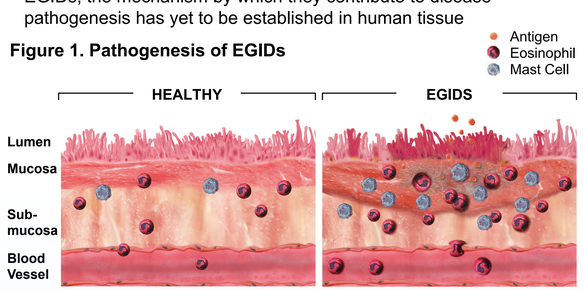
BACKGROUND

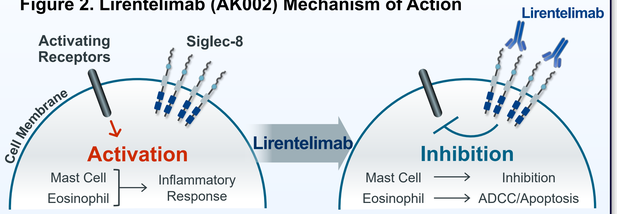
- Eosinophilic gastrointestinal diseases (EGIDs) are a rare set of conditions characterized by the pathologic accumulation of eosinophils in the gastrointestinal tract
- While eosinophils have been strongly associated with EGIDs, localized mast cells are also elevated in eosinophilic esophagitis, gastritis, and duodenitis
- Despite evidence of mast cells being an important component of EGIDs, the mechanism by which they contribute to disease pathogenesis has yet to be established in human tissue

Figure 1. Pathogenesis of EGIDs



- Current treatment options such as diet restriction and corticosteroids have limited efficacy and/or are inappropriate for chronic use
- There is a significant unmet need for novel therapies

Figure 2. Lirentelimab (AK002) Mechanism of Action



- Siglec-8 is an inhibitory receptor selectively expressed on human eosinophils and mast cells, and therefore represents a novel target for the treatment of EGIDs
- Lirentelimab is a novel, humanized, non-fucosylated IgG1 monoclonal antibody to Siglec-8 that depletes blood and tissue eosinophils and broadly inhibits mast cell degranulation and cytokine production
- Lirentelimab has recently demonstrated significant symptomatic and histological improvement in a multi-center, randomized, double-blind placebo-controlled Phase 2 study in patients with eosinophilic gastritis and/or gastroenteritis

METHODS

- Single-cell suspensions were prepared by enzymatic & mechanical digestion (Figure 3) of fresh biopsies from patients clinically diagnosed with EoE or non-disease control esophageal tissue
- Multi-color flow cytometry was performed to quantify immune cells and evaluate the activation state of eosinophils & mast cells as shown in Figure 4
- Mast cells were FACS-sorted from EoE biopsies or non-diseased GI tissues as shown in Figure 7 followed by overnight incubation with or without PMA/Ionomycin
- Cell-free supernatants were collected the following day and cytokines were quantified using meso scale discovery (MSD) system
- The following cytokines were analyzed: IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-18, IL-33, GM-CSF, INFγ, TNFα, CCL2, CCL3, CCL4, CCL11, CCL17, and VEGF

Figure 3. Study Design

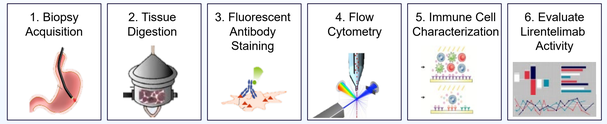
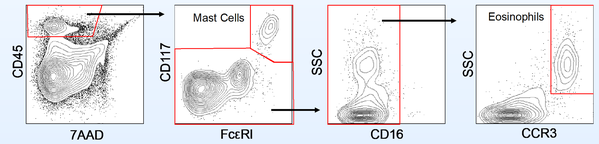


Figure 4. Flow Cytometry Gating Strategy for Mast Cells and Eosinophils in EoE Biopsy Tissue



RESULTS

Figure 5. Increased Numbers of Eosinophils and Mast Cells in EoE Biopsies

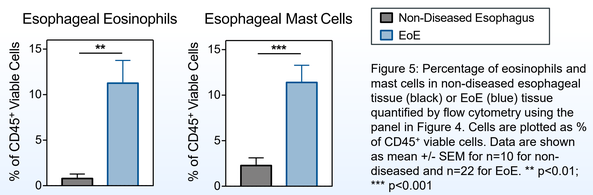


Figure 5: Percentage of eosinophils and mast cells in non-diseased esophageal tissue (black) or EoE (blue) tissue quantified by flow cytometry using the panel in Figure 4. Cells are plotted as % of CD45$^+$ viable cells. Data are shown as mean +/- SEM for n=10 for non-diseased and n=22 for EoE. ** p<0.01; *** p<0.001

Figure 6. Resting Mast Cells Display an Increased Activation State in EoE Biopsies

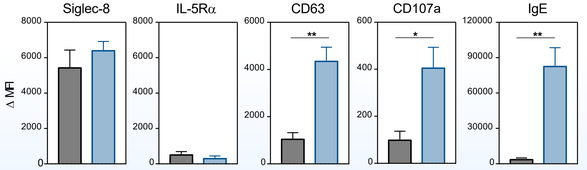


Figure 6: Expression of surface markers on mast cells were determined by flow cytometry in EoE biopsies (blue) or non-diseased esophageal tissue (black). Mast cells were gated on viable, CD45$^+$, CD117$^+$, FcεRI$^+$ and ΔMFI was calculated by subtracting MFI from a negative FMO control. Data are shown as mean +/- SEM for n=5 for non-diseased and n=10 for EoE.
* p<0.05; ** p<0.01

Figure 7. Gating Strategy and Method for Activating Sorted Mast Cells from GI Tissue

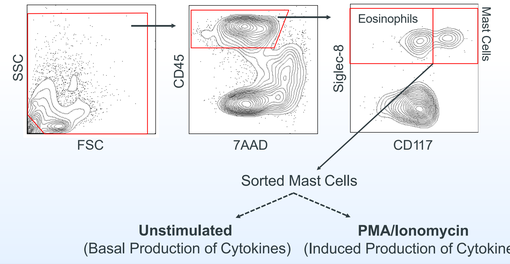


Figure 8. Mast Cells from EoE Tissue Basally Produce IL-5, IL-13, and CCL3

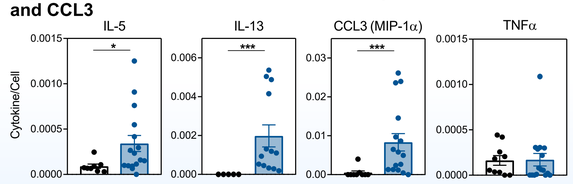


Figure 8: Cytokine level per mast cell in supernatant from overnight culture of unstimulated FACS-sorted mast cells from EoE biopsies (blue) or non-diseased GI tissue (black). Cytokine/cell was calculated by dividing the cytokine concentration (pg/mL) by total mast cells plated. Individual donors are plotted. * p<0.05; *** p<0.001

Figure 9. EoE Tissue Mast Cells Produce Increased Levels of Cytokines upon Stimulation with PMA/Ionomycin

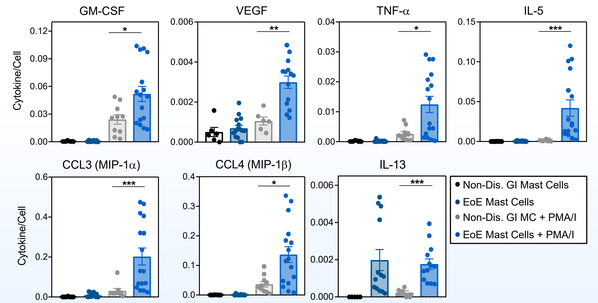


Figure 9: Cytokine level per mast cell in supernatant from overnight incubation of unstimulated or PMA-Ionomycin activated, FACS-sorted mast cells from EoE biopsies (blue) or non-diseased GI tissue (black). Cytokine/cell was calculated by dividing the cytokine concentration (pg/mL) by total mast cells plated.
* p<0.05; ** p<0.01; *** p<0.001

Figure 10. Mast Cell-Derived GM-CSF and VEGF Correlate with Tissue Eosinophil Percentage in EoE Biopsies

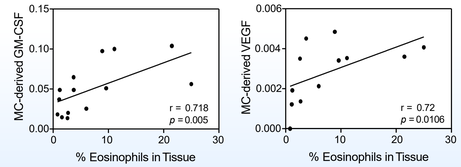


Figure 10: Correlation analysis (Spearman) of GM-CSF and VEGF cytokine levels/cell from activated EoE FACS-sorted mast cells and the percentage of tissue eosinophils in the EoE biopsies

CONCLUSIONS

- Elevated and activated mast cells are found in patients with EoE
- Mast cells from EoE tissue basally produced type 2 cytokines that are associated with T cell activation and eosinophilic inflammation
- Upon stimulation, EoE mast cells further produced abundant cytokines and chemokines that correlated with the percentage of tissue eosinophils, suggesting mast cells can directly recruit eosinophils to inflamed tissue
- Therefore, targeting both eosinophils and mast cells may be needed to significantly reduce inflammation

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Reference: (1) Caldwell JM, et al. J Allergy Clin Immunol. 2014.; (2) Youngblood BA, et al. JCI Insights. 2019.; (3) Jensen ET, et al. J Pediatr Gastroenterol Nutr. 2016.