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

Melina Butuci¹, Emily C. Brock¹, Julia Schanan¹, Alan Xu¹, Henrik S. Rasmussen¹, Bhupinder Singh¹, Richard Drake², Amy Holman², Kathryn Peterson², and Bradford A. Youngblood¹

¹Alatas Inc. Redwood City, CA; ²University of Utah, UT

Figures

1. Pathogenesis of EGIDs

2. Tissue Eosinophil Percentage in EoE Biopsies

3. Study Design

4. Flow Cytometry Gating Strategy for Mast Cells and Eosinophils in EoE Biopsies

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

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

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

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

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

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

Results

- 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 measured using enzyme-linked immunosorbent assays (ELISA)

Methods

- Patients with EoE (n=12) and healthy controls (n=12) were studied
- Biopsies were obtained from the esophagus and processed for flow cytometry
- Flow cytometry was performed using a variety of antibodies to detect surface markers and intracellular cytokines
- Data were analyzed using the GraphPad Prism software

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 directly recruit eosinophils to inflamed tissue
- Therefore, targeting both eosinophils and mast cells may be needed to significantly reduce inflammation