Eosinophilic Esophagitis and Gastritis Patient Biopsies Have High Levels of Activated Eosinophils and Mast Cells That Are Inhibited by AK002

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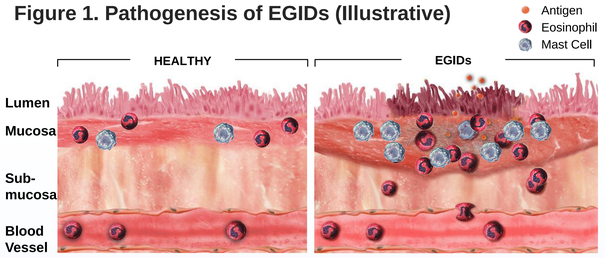
BACKGROUND

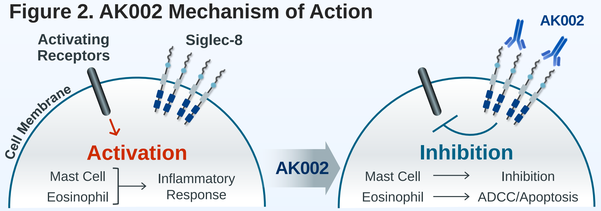
- Pathologic accumulation and over-activation of eosinophils are implicated in multiple chronic inflammatory diseases in the GI tract including eosinophilic esophagitis (EoE), gastritis (EG), gastroenteritis (EGE), and colitis - collectively termed eosinophilic gastrointestinal disorders (EGIDs)
- Patients with EGIDs have decreased quality of life due to debilitating symptoms such as dysphagia, abdominal pain, nausea, vomiting, and diarrhea
- While the pathogenesis of EGIDs has historically been thought to be driven by eosinophils, mast cells have also been shown to be elevated in EGIDs[1]
- Despite the strong association of mast cells in EGIDs, no further characterization of these cells has been performed

Figure 1. Pathogenesis of EGIDs (Illustrative)



- EG and EGE affect 45,000 - 50,000 patients in the US, though this number may be significantly underestimated[2]
- 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. 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
- AK002 is a novel, humanized, non-fucosylated IgG1 monoclonal antibody to Siglec-8
- Engagement of Siglec-8 receptor by AK002 triggers:
  - Antibody dependent cell mediated cytotoxicity (ADCC) against eosinophils (blood)
  - Inhibition of mast cells and apoptosis of tissue eosinophils (tissue)
- Surface phenotyping of eosinophils and mast cells, and the activity of AK002 have not been previously evaluated in EGID tissue

METHODS

- Single-cell suspensions were prepared by enzymatic & mechanical digestion (Miltenyi) of fresh biopsies from patients clinically diagnosed with EGIDs (n=12) or non-disease controls (n=12)
- Multi-color flow cytometry was performed to quantify immune cells and evaluate the activation state of eosinophils & mast cells
- Ex vivo activity of AK002 was evaluated against eosinophils and mast cells in dissociated EGID biopsies using Luminex (Millipore)

Figure 3. Study Design

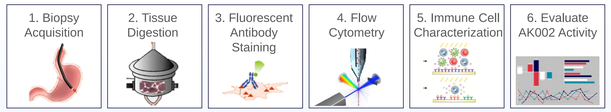
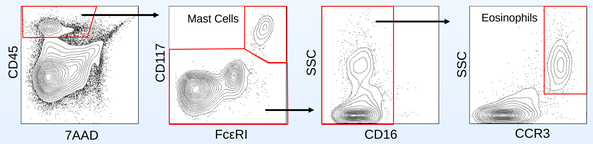


Figure 4. Flow Cytometry Gating Strategy for Mast Cells and Eosinophils in EoE and EG Biopsy Tissue



RESULTS

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

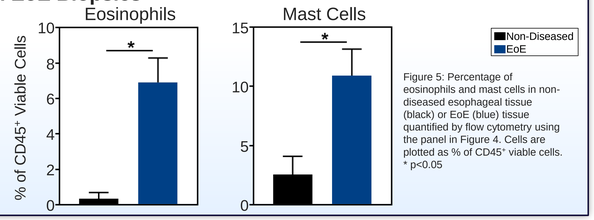


Figure 5: Percentage of eosinophils and mast cells in non-diseased esophageal tissue (black) or EoE (blue) tissue quantified by flow cytometry using the panel in Figure 4. Cells are plotted as % of CD45⁺ viable cells. * p<0.05

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

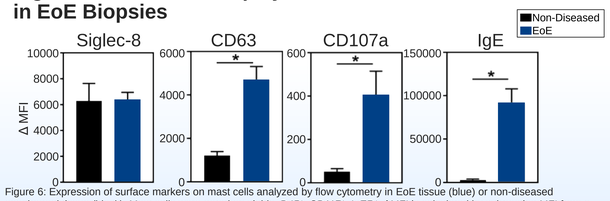


Figure 6: Expression of surface markers on mast cells analyzed by flow cytometry in EoE tissue (blue) or non-diseased esophageal tissue (black). Mast cells were gated on viable, D45⁺, CD117⁺, IgER⁺. ΔMFI is calculated by subtracting MFI from a negative FMO control. * p<0.05

Figure 7. Increased Numbers of Eosinophils and Mast Cells in EG Biopsies

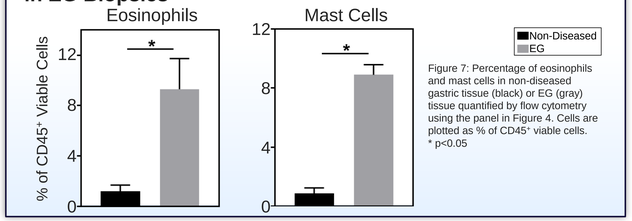


Figure 7: Percentage of eosinophils and mast cells in non-diseased gastric tissue (black) or EG (gray) tissue quantified by flow cytometry using the panel in Figure 4. Cells are plotted as % of CD45⁺ viable cells. * p<0.05

Figure 8. Eosinophils Are Highly Activated in EG Patients

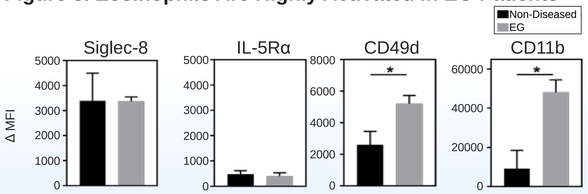


Figure 8: Expression of surface markers on eosinophils determined by flow cytometry in EG biopsies (gray) or non-diseased gastric tissue (black). Eosinophils were gated on viable, CD45⁺, SSCHi, CD16⁻, CCR3⁺ and ΔMFI was calculated by subtracting MFI from a negative FMO control. * p<0.05

Figure 9. Mast Cells Display an Increased Activation State in EG Biopsies

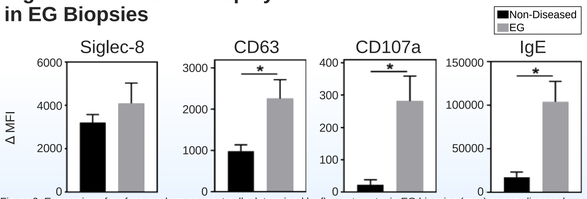


Figure 9: Expression of surface markers on mast cells determined by flow cytometry in EG biopsies (gray) or non-diseased gastric tissue (black). Mast cells were gated on viable, CD45⁺, CD117⁺, IgER⁺ and ΔMFI is calculated by subtracting MFI from a negative FMO control. * p<0.05

Figure 10. Siglec-8 is Selectively Expressed on Eosinophils and Mast Cells in EoE and EG Human Tissue

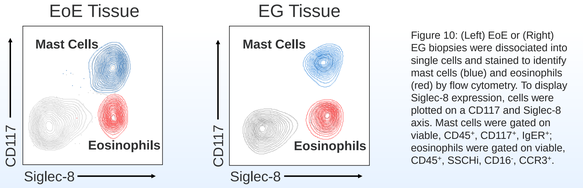


Figure 10: (Left) EoE or (Right) EG biopsies were dissociated into single cells and stained to identify mast cells (blue) and eosinophils (red) by flow cytometry. To display Siglec-8 expression, cells were plotted on a CD117 and Siglec-8 axis. Mast cells were gated on viable, CD45⁺, CD117⁺, IgER⁺; eosinophils were gated on viable, CD45⁺, SSCHi, CD16⁻, CCR3⁺.

Figure 11. AK002 Inhibits IgE-mediated Mast Cell Mediator Production in ex vivo EG Biopsies

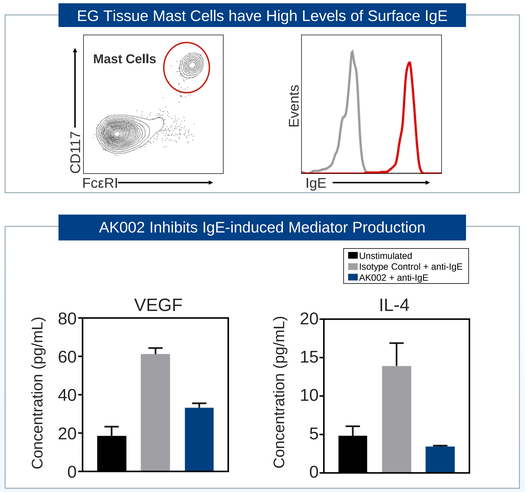


Figure 11: (Top) Identification of mast cells in EG dissociated biopsy by flow cytometry and expression of surface-bound IgE on mast cells (red) compared to an FMO negative control (gray). (Bottom) EG dissociated biopsies were cultured overnight in an unstimulated state (black) or in the presence an anti-IgE Ab to crosslink FcεRI on mast cells. Cells activated with the anti-IgE antibody were also cultured overnight with AK002 (blue) or an isotype control (gray) followed by analysis of VEGF and IL-4 in the cell culture supernatants after 24 hours.

- EG biopsies contain an abundant IgE-primed mast cell population
- Crosslinking of the IgE receptor on mast cells results in increased VEGF and IL-4 production in dissociated EG biopsies
  - VEGF and IL-4 play a role in recruiting inflammatory cells such as eosinophils
- AK002 inhibits IgE-mediated VEGF and IL-4 production in dissociated EG biopsies

CONCLUSIONS

- Eosinophil and mast cell numbers are significantly increased in biopsy samples from patients diagnosed with EoE and EG
- Mast cells are elevated to the same extent as eosinophils in both EoE and EG biopsies
- Eosinophils and mast cells in EG biopsies display an activated phenotype, suggesting they may be pathogenic in EGIDs
- Siglec-8 is selectively expressed on eosinophils and mast cells at high levels in EGID biopsies
- AK002, a humanized Siglec-8 antibody, demonstrates anti-eosinophilic and mast cell inhibitory activity in EG biopsies
- Targeting Siglec-8 with AK002 may represent a novel approach to treat EGIDs

Acknowledgements:
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References:
1)Caldwell et al. J Allergy Clin Immunol. 2014 Nov;134(5):1114-24.
2)Jensen ET, et al. J Pediatr Gastroenterol Nutr. 2016 Jan;62(1):36-42.