Mast Cells Are Significantly Activated In Patients with Ulcerative Colitis and Are Inhibited by an Anti-Siglec-8 Antibody, Antolimab (AK002)

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BACKGROUND

- Accumulation and activation of mast cells and eosinophils have been implicated in the pathogenesis of several chronic inflammatory gastrointestinal (GI) diseases, including eosinophilic gastrointestinal diseases (EGIDs) and inflammatory bowel disease (IBD)[1]
- Despite the strong association of mast cells and eosinophils in IBD, no further characterization of these cells has been performed
- Here, we evaluated the activation state of mast cells and eosinophils and quantified the production of cytokines from mast cells in colon tissue from IBD or non-diseased control patients

Figure 1. Mast Cells and Eosinophils are Key Drivers of Acute and Chronic Inflammation

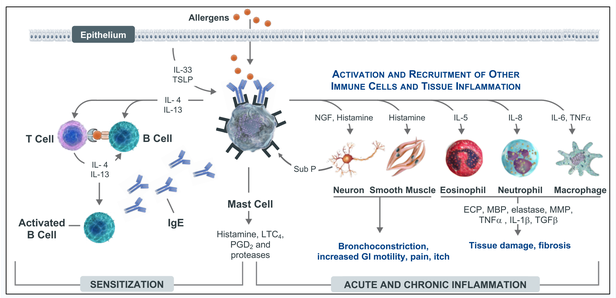
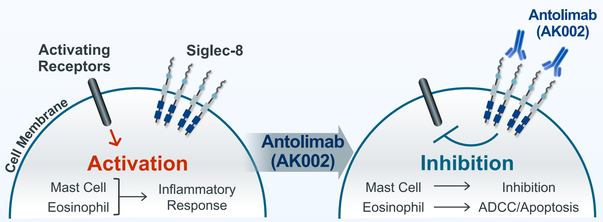


Figure 2. Antolimab (AK002) Mechanism of Action



- Siglec-8 is an inhibitory receptor selectively expressed on human eosinophils and mast cells and represents a target for the treatment of IBD
- Antolimab (AK002) is a humanized, non-fucosylated IgG1 monoclonal antibody to Siglec-8 that depletes blood eosinophils by antibody dependent cellular cytotoxicity (ADCC) and induces apoptosis of tissue eosinophils
- In addition, antolimab inhibits both IgE-dependent and independent modes of mast cell activation
- Antolimab has recently demonstrated significant symptomatic and histological improvement in a randomized, double-blind, placebo-controlled Phase 2 study in patients with eosinophilic gastritis and/or duodenitis

METHODS

- Single-cell suspensions were prepared by enzymatic digestion of fresh colon biopsies from patients with IBD or non-diseased control colon tissue
- Multi-color flow cytometry was performed to identify and evaluate the activation state of mast cells and eosinophils
- Mast cells were FACS-sorted from ulcerative colitis biopsies or non-diseased human colon tissue to evaluate cytokine production after overnight stimulation with PMA/Ionomycin
- The inhibitory activity of antolimab was evaluated using human colon tissue mast cells stimulated with LPS

Figure 3. Study Design

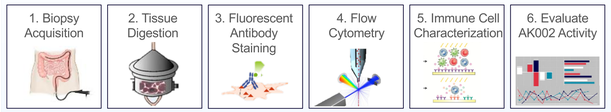
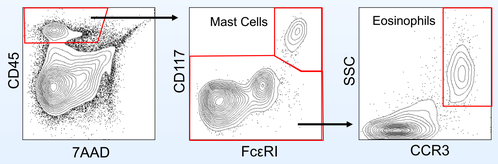


Figure 4. Flow Cytometry Gating Strategy for Mast Cells and Eosinophils in IBD Biopsy Tissue



RESULTS

Figure 5. Mast Cells and Eosinophils are Significantly Elevated in Ulcerative Colitis Biopsies

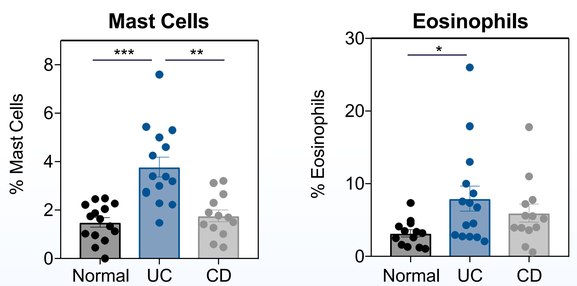


Figure 5: Percentage of colonic tissue mast cells and eosinophils in biopsy tissue from UC and CD patients or non-diseased control colon tissue. Mast cells and eosinophils were quantified by flow cytometry as shown in Figure 4. Graphs are plotted as the percentage of CD45+ viable cells from individual patients +/- SEM. * p=<0.05, ** p=<0.01, *** p=<0.001 determined by one-way ANOVA with Tukey's multiple comparisons test

- The percentage of mast cells was significantly increased in ulcerative colitis (UC) biopsy tissue compared to Crohn's disease (CD) and non-diseased colon tissue
- In addition, the percentage of eosinophils was significantly increased in UC and nominally elevated in CD biopsy tissue compared to non-diseased colon tissue

Figure 6. Mast Cells in Ulcerative Colitis Tissue Display an Increased Degranulation and Activation State

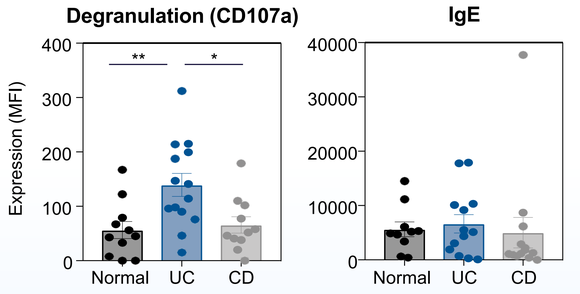


Figure 6: Expression of the mast cell degranulation marker (CD107a) and surface bound IgE on mast cells in biopsy tissue from UC and CD patients or non-diseased control colon tissue. Data are shown as the mean fluorescence intensity (MFI) from individual patients +/- SEM. * p=<0.05, ** p=<0.01

- The expression of the mast cell degranulation marker CD107a was significantly increased on mast cells from UC biopsy tissue compared to CD and non-diseased colon tissue mast cells
- However, unlike mast cells in allergic disease, IgE expression was unchanged between UC, CD, and non-diseased colon mast cells which suggest a non-IgE-driven mechanism of mast cell activation in IBD

Figure 7. Eosinophils in Ulcerative Colitis and Crohn's Disease Tissue Display an Increased Activation State

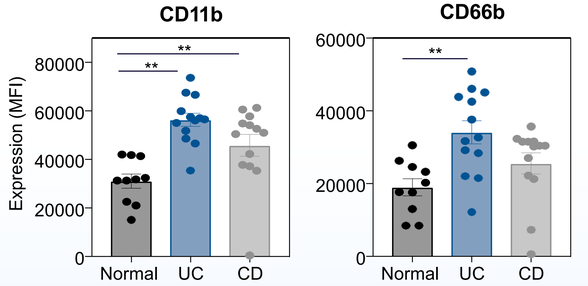


Figure 7: Expression of the eosinophil activation markers, CD11b and CD66b on eosinophils in biopsy tissues from UC and CD patients or non-diseased control colon tissue. Data are shown as the mean fluorescence intensity (MFI) from individual patients +/- SEM. * p=<0.05, ** p=<0.01

- The expression of the eosinophil activation marker CD11b was significantly increased on both UC and CD tissue eosinophils compared to non-diseased colon tissue eosinophils
- UC biopsy tissue eosinophils also displayed significantly increased expression of CD66b compared to non-diseased colon tissue eosinophils

Figure 8. Mast Cells from Ulcerative Colitis Tissue Produce Higher Levels of TNF, IL-5, and CCL3 Compared to Normal Colon Tissue Mast Cells

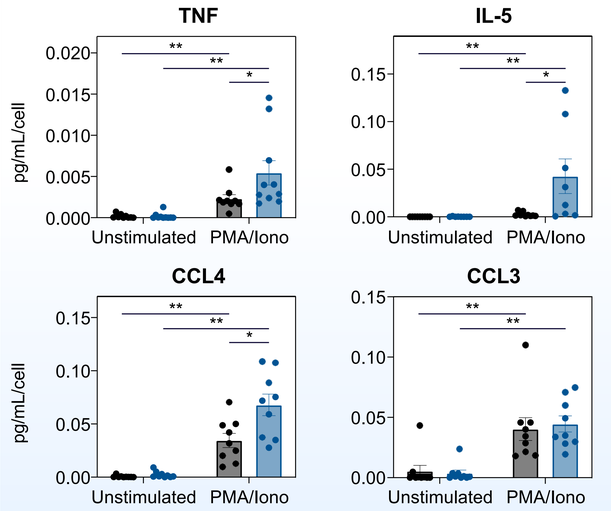


Figure 8: Mast cells were sorted by FACS from either non-diseased colon tissue (black) ot UC tissue (blue) and cultured overnight unstimulated or with PMA/Ionomycin followed by cytokine analysis in the supernatant. Data are shown as pg/mL/sorted cell number from individual donors +/- SEM. * p=<0.05, ** p=<0.01

Figure 9. Antolimab (AK002) Suppresses Cytokine Production from Human GI Tissue Mast Cells

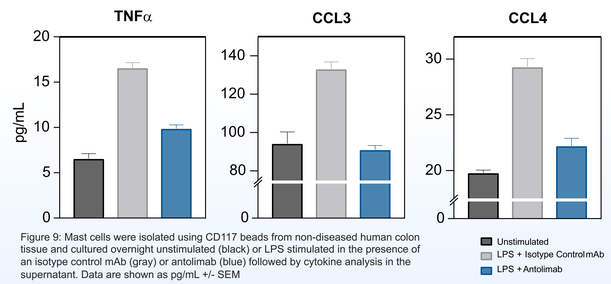


Figure 9: Mast cells were isolated using CD117 beads from non-diseased human colon tissue and cultured overnight unstimulated (black) or LPS stimulated in the presence of an isotype control mAb (gray) or antolimab (blue) followed by cytokine analysis in the supernatant. Data are shown as pg/mL +/- SEM

- Antolimab reduces cytokines associated with driving IBD through mast cell inhibition

CONCLUSIONS/DISCUSSION

- Mast cells and eosinophils may play a significant role in driving the pathogenesis of ulcerative colitis
- Ulcerative colitis tissue mast cells produce increased levels of disease-driving mediators, including TNFα that are inhibited by antolimab (AK002)
- Antolimab may represent a potential targeted therapy in IBD

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Reference: 1) Hamilton, M.J., et al Inflamm Bowel Dis. 2014 December ; 20(12): 2364–2378