Mast Cells are Locally Activated and Express Functional MRGPRX2 in Biopsies from Symptomatic Eosinophilic Gastritis and Duodenitis Patients

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BACKGROUND

- Eosinophilic gastritis (EG) and/or duodenitis (EoD) are associated with the pathologic accumulation and activation of eosinophils (Eos) and mast cells (MCs) in the stomach and/or duodenum (Figure 1)[1,2]
- Patients with EG and/or EoD have decreased quality of life due to chronic debilitating and often nonspecific symptoms such as abdominal pain, abdominal cramping, bloating, early satiety, loss of appetite, nausea, vomiting, & diarrhea
- 45% of patients with moderate-severe chronic GI symptoms and/or history of functional GI diagnoses met histologic criteria for EG and/or EoD, suggesting EG and/or EoD may be significantly underdiagnosed[3]

Figure 1. Pathogenesis of EGIDs

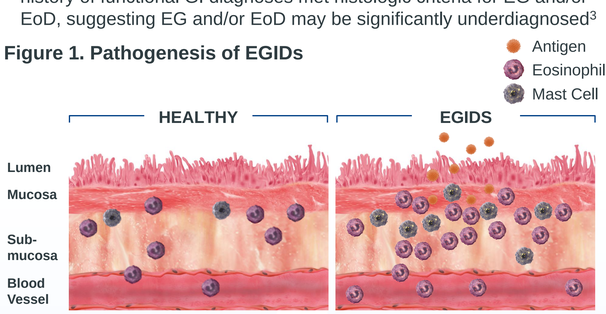
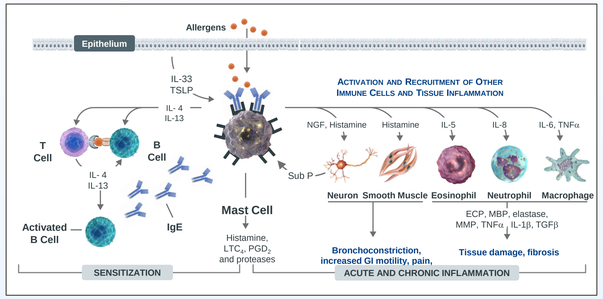


Figure 2. Mast Cells and Eosinophils Are Key Drivers of Inflammatory Disease

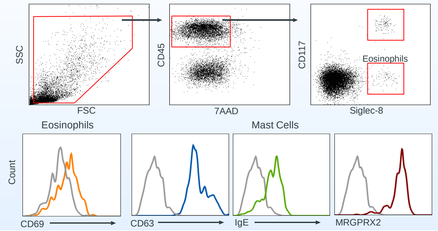


- MCs are tissue-resident immune cells that regulate acute and chronic inflammation through IgE-dependent and –independent mechanisms (Figure 2)
- IgE-dependent MC activation through FcεRI is a known driver of allergic diseases, however, IgE-independent activation, particularly via MRGPRX2, is now recognized as an important regulator of pain and allergic inflammation
- Although Eos are recognized as key effector cells in EG and/or EoD, the immunological mechanisms that contribute to eosinophilic inflammation and non-specific GI symptoms are unknown

METHODS

- Gastric and duodenal biopsies were obtained from EG and/or EoD patients meeting predefined moderate-severity symptom criteria and healthy controls (HC) i.e. non-diseased subjects with minimal or no symptoms
- Flow cytometry and bulk RNA-sequencing were used to phenotype tissue MCs and eosinophils in GI biopsies
- Levels of inflammatory mediators were measured in whole GI tissue ex vivo biopsy supernatants after overnight culture

Figure 3. Strategy to Identify and Phenotype Eos and MCs in Gastric and Duodenal Biopsies



RESULTS

Figure 4. Eos and MCs are Elevated in Symptomatic EG and/or EoD patients compared to Healthy Controls

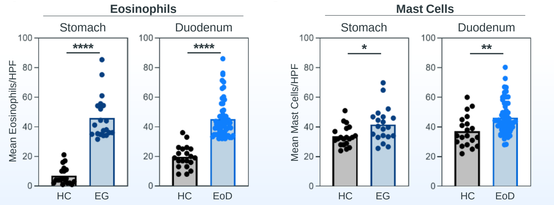


Figure 4: Eosinophil and MC counts (mean/HPF) in gastric and duodenal biopsies from healthy controls (black) or symptomatic patients with gastric disease (EG only, EG+EoD, dark blue) and/or duodenal disease (EoD only, EG+EoD, light blue). Data are plotted as mean +/- SEM (n=20 controls; n=22 EG/EoD with gastric disease; n=67 EG/EoD with duodenal disease). * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

Figure 5. Eos and MCs are Activated in EG and/or EoD Gastric & Duodenal Biopsies Compared to Controls

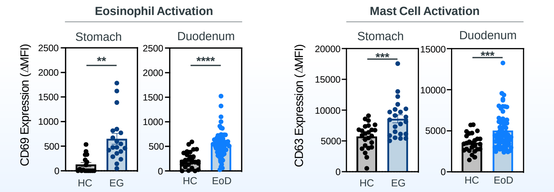


Figure 5: Surface expression of CD69 and CD63 in gastric and duodenal biopsies from healthy controls (black) or symptomatic patients with gastric disease (EG only, EG+EoD, dark blue) and/or duodenal disease (EoD only, EG+EoD, light blue). Data are plotted as mean +/- SEM (n=20 controls; n=22 EG/EoD with gastric disease; n=67 EG/EoD with duodenal disease). * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

Figure 6. Eosinophil and MC Mediators are Locally Elevated in EG and/or EoD Biopsy Supernatants compared to Healthy Controls

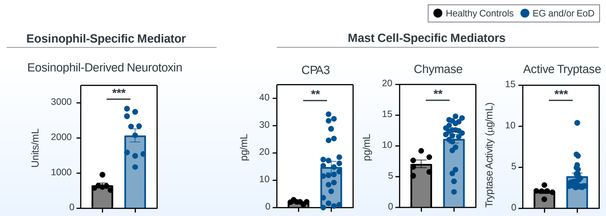


Figure 6: Levels of EDN or MC proteases in in gastric and duodenal biopsies from healthy controls (black) or symptomatic patients with EG and/or EoD (blue). Data are plotted as mean +/- SEM (n=7-8 controls; n=13 EG/EoD with gastric disease; n=19 EG/EoD with duodenal disease). * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

Figure 7. MRGPRX2 is Elevated and Functional on MCs from EG and/or EoD Biopsies

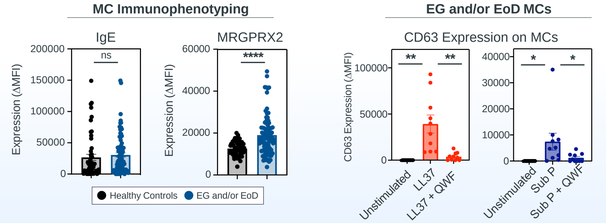


Figure 7: (Left) Surface expression of IgE or MRGPRX2 on MCs in GI biopsies from healthy controls (black) or symptomatic patients with EG and/or EoD (blue). (Right) CD63 expression on MCs from EG/EoD biopsies stimulated LL37 or Substance P or in the presence of QWF (antagonist) for 30 minutes.

Figure 8. Eosinophil Chemokines and Cytokines, and MRGPRX2 Ligands are Locally Elevated in EG and/or EoD Biopsy Supernatants

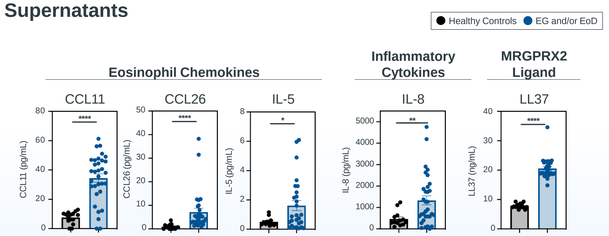


Figure 8: Levels of eosinophil-attracting chemokines, inflammatory cytokines, or MRGPRX2 ligand in cell-free supernatants from overnight ex vivo cultured GI biopsies from healthy controls (black) or symptomatic patients with EG and/or EoD (blue). Data are plotted as mean +/- SEM (n=12-15 controls; n=28-32 EG/EoD patients) * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

- Biopsies from symptomatic EG and/or EoD patients produced significantly elevated levels of Eos chemokines and inflammatory cytokines, consistent with local tissue inflammation
- In addition, levels of the MRGPRX2 ligand, LL37 were elevated in biopsies from EG and or/EoD patients compared to Healthy Controls

Figure 9. LL37 Induces MC Activation and Production of Eosinophil and Inflammatory Cytokines in EG and/or EoD Biopsies

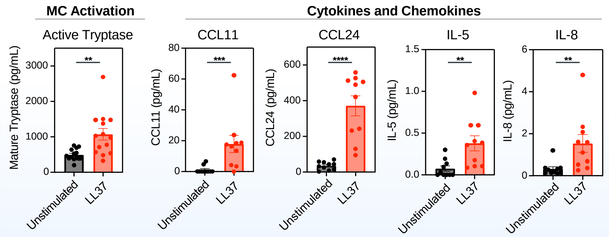


Figure 9: Ex vivo EG and/or EoD biopsies were cultured unstimulated or stimulated with LL37 overnight followed by quantification of active tryptase or cytokines and chemokines in the supernatant. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

- MRGPRX2-mediated MC activation using LL37 induces production of eosinophil and inflammatory mediators in EG and/or EoD biopsies

Figure 10. MRGRX2-mediated MC Activation Induces Migration of Human Eosinophils

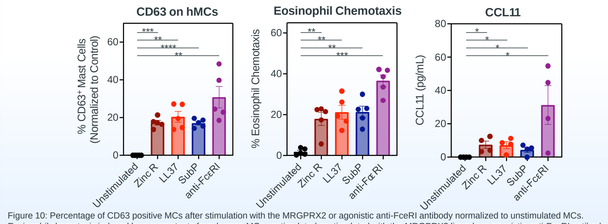


Figure 10: Percentage of CD63 positive MCs after stimulation with the MRGPRX2 or agonistic anti-FcεRI antibody normalized to unstimulated MCs. Eosinophil chemotaxis induced by supernatants from human MCs unstimulated or stimulated with the MRGPRX2 ligands or agonistic anti-FcεRI antibody normalized. Levels of eosinophil-attracting chemokines in supernatants of stimulated human MCs. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001

CONCLUSIONS/DISCUSSION

- Eos and MCs in gastric and duodenal biopsies from EG only, EoD only, and EG+EoD patients with moderate-severe GI symptoms are significantly elevated and locally activated
- Symptomatic EG and/or EoD patients display significant local gastric and duodenal inflammation characterized by elevated levels of Eos chemokines, inflammatory cytokines, and MRGPRX2 ligand
- The MC-specific neuropeptide receptor, MRGPRX2 is elevated and functional on EG and/or EoD MCs, and activation via endogenous ligands induces eosinophilic inflammation, suggesting IgE-independent MC activation may contribute to EGID pathogenesis
- These data demonstrate that MC activation via MRGPRX2 is a GI disease-relevant mechanism that contributes to EG/EoD

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Reference: (1) Caldwell JM, et al. J Allergy Clin Immunol. 2014.; (2) Youngblood BA, et al. Gastroenterology. 2019.; (3) Jensen ET, et al. J Pediatr Gastroenterol Nutr. 2016.; (4) Licari AL, et al. JACI. 2020.; (5) Dellon ES, et al. NEJM. 2020.