Activated Mast Cells and Eosinophils are Associated with Increased Inflammatory Mediators in Mucosal Biopsies from EG and/or EoD Patients with Chronic Gastrointestinal Symptoms

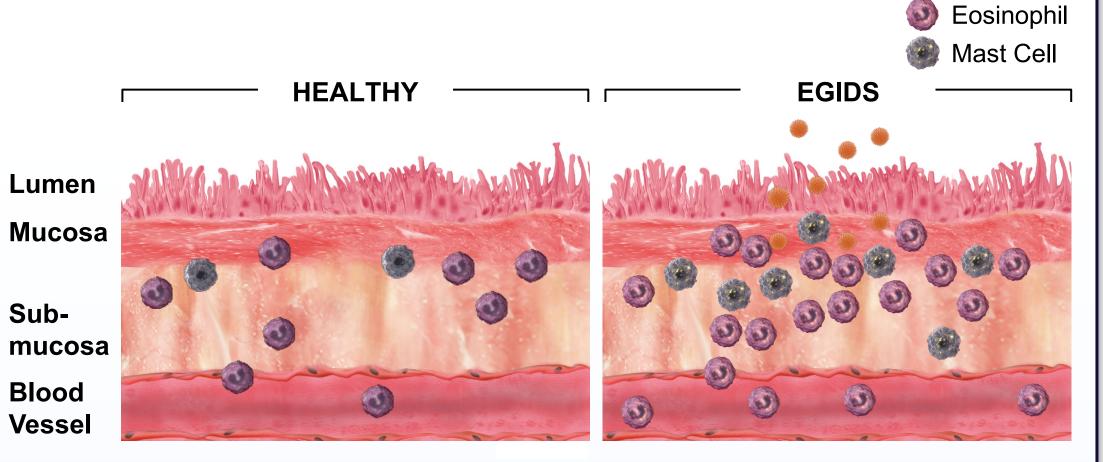
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BACKGROUND

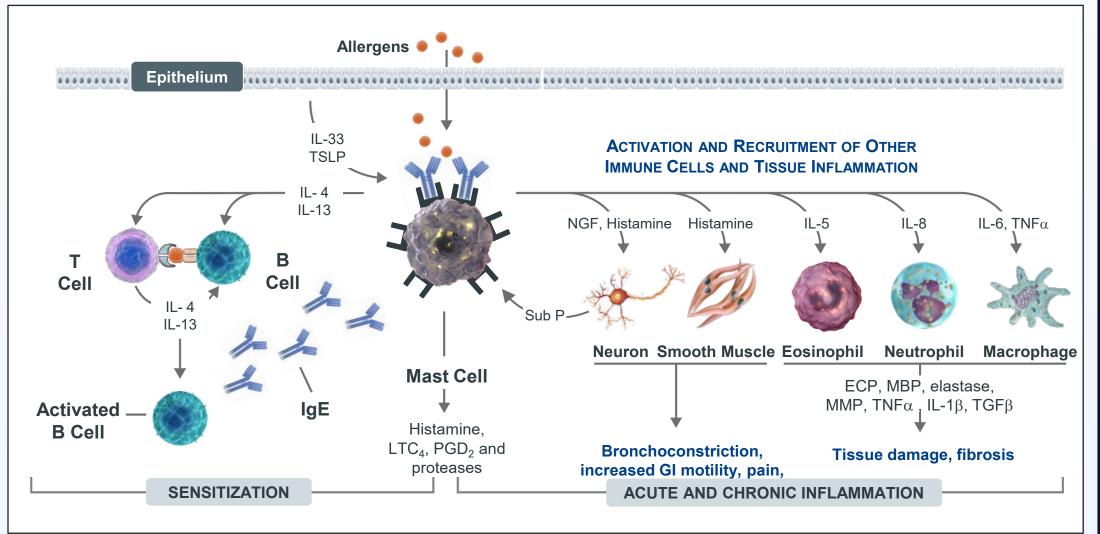
- Eosinophilic gastritis (EG) and/or duodenitis (EoD) are associated with the pathologic accumulation and activation of eosinophils (Eos) and mast cells (MCs) in the stomach and/or duodenum (Figure 1)^{1,2}
- Patients with EG and/or EoD have decreased quality of life due to chronic debilitating and often nonspecific symptoms such as abdominal pain, abdominal cramping, bloating, early satiety, loss of appetite, nausea, vomiting, & diarrhea
- Recently, a large prospective study demonstrated that a meaningful proportion of patients with 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 underdiagnosed³





Antigen

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

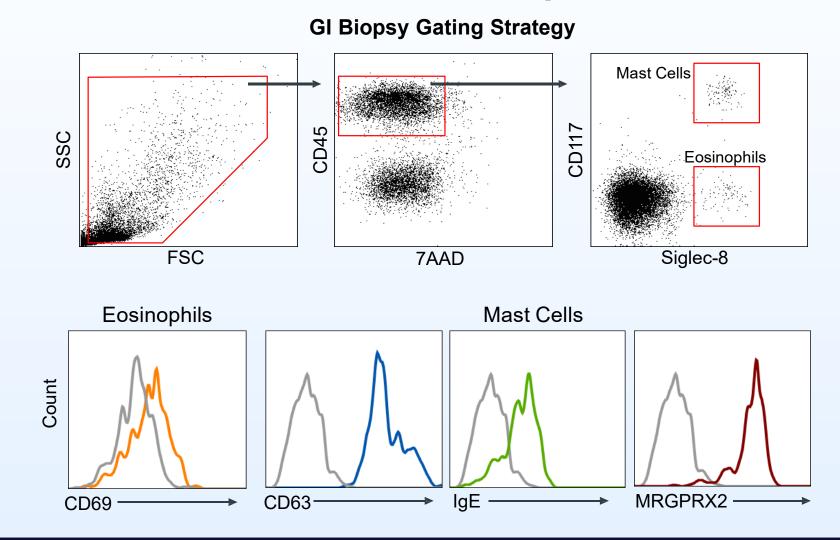


- MCs are tissue-resident immune cells that regulate acute and chronic inflammation through IgE-dependent and – independent mechanisms (Figure 2)
- IgE-dependent MC activation through FceRI is a known driver of allergic diseases, however, IgE-independent activation, particularly via MRGPRX2, is now recognized 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

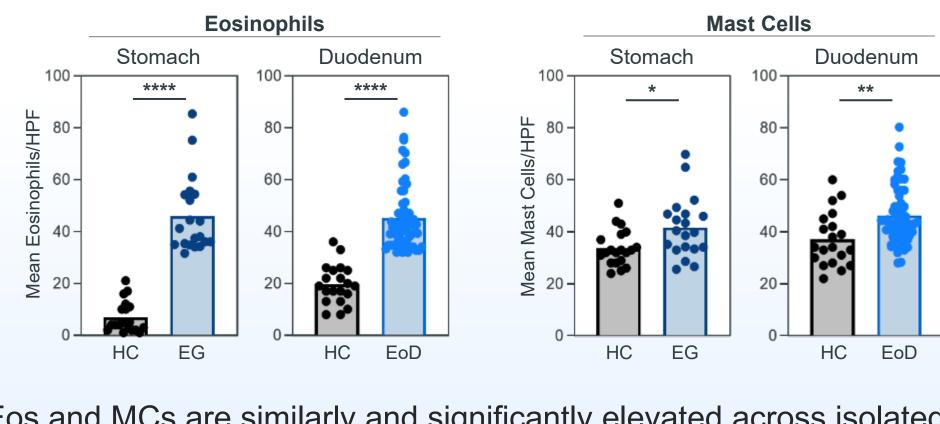
- Gastric and duodenal biopsies were obtained from EG and/or EoD patients meeting pre-defined symptom severity criteria and non-diseased subjects with minimal or no symptoms
- Flow cytometry and bulk RNA-sequencing were used to phenotype tissue MCs and eosinophils in GI biopsies
- Levels of inflammatory 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

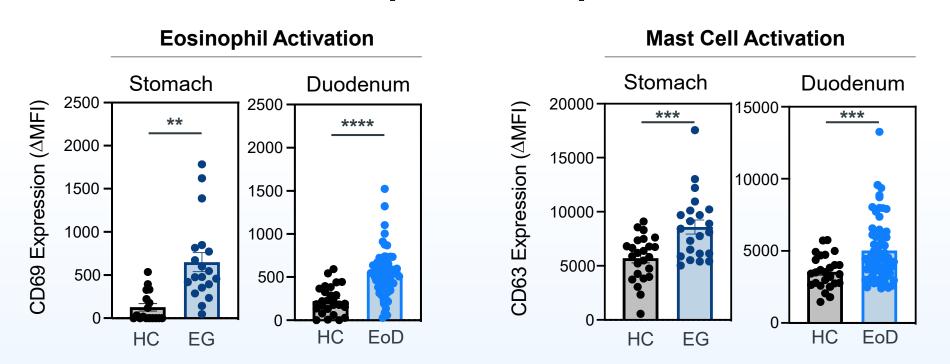
Figure 4. Eos and MCs are Elevated in Symptomatic EG and/or EoD patients compared to Healthy Controls



Eos and MCs are similarly and significantly elevated across isolated and overlapping EG and/or EoD

* p <0.05; ** p<0.01; *** p<0.0001; **** p<0.0001

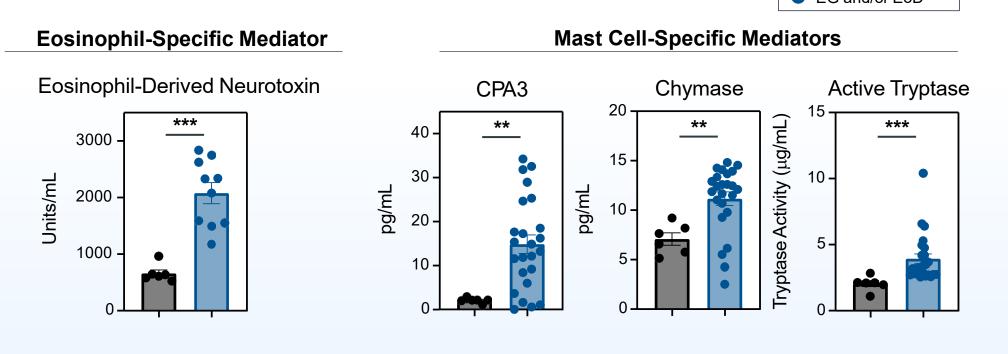
Figure 5. Eos and MCs are Activated in EG and/or EoD Gastric & Duodenal Biopsies Compared to Controls



Eos and MCs are similarly and significantly activated across both isolated and overlapping EG and/or EoD, as demonstrated by expression of the surface activation markers CD69 and CD63

* p <0.05; ** p<0.01; *** p<0.0001; **** p<0.0001

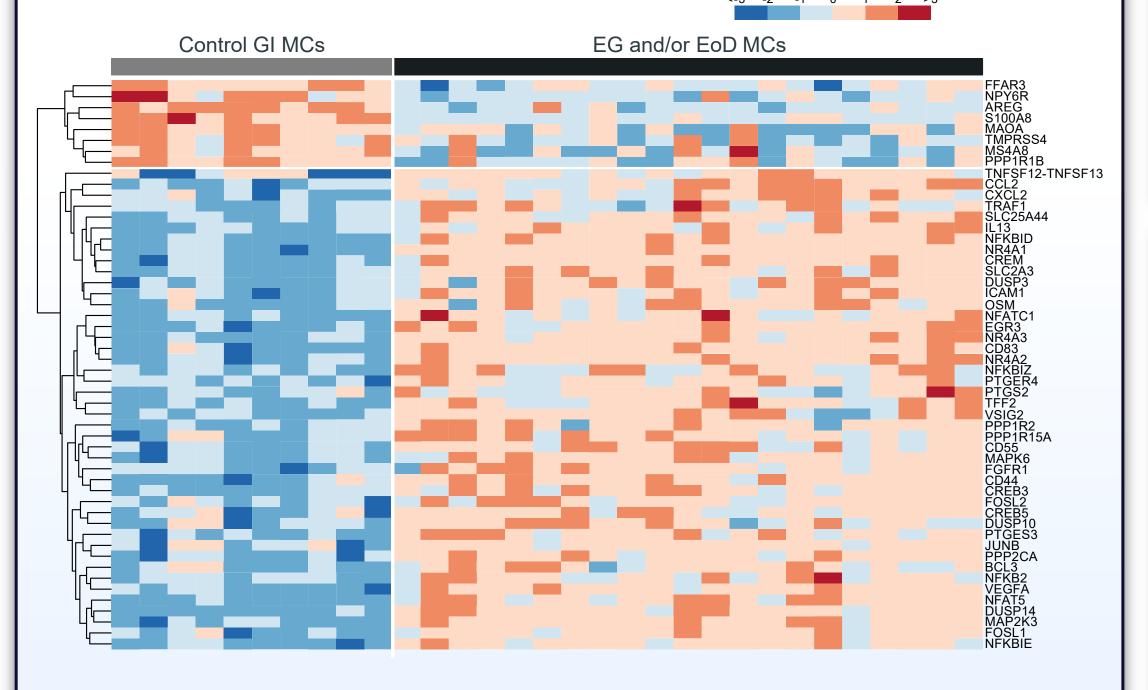
Figure 6. Eosinophil and MC Mediators are Locally Elevated in EG and/or EoD Biopsy Supernatants compared to Healthy Controls



 Symptomatic EG and/or EoD patients have functionally activated Eos and MCs in local tissue compared to Healthy Control biopsies

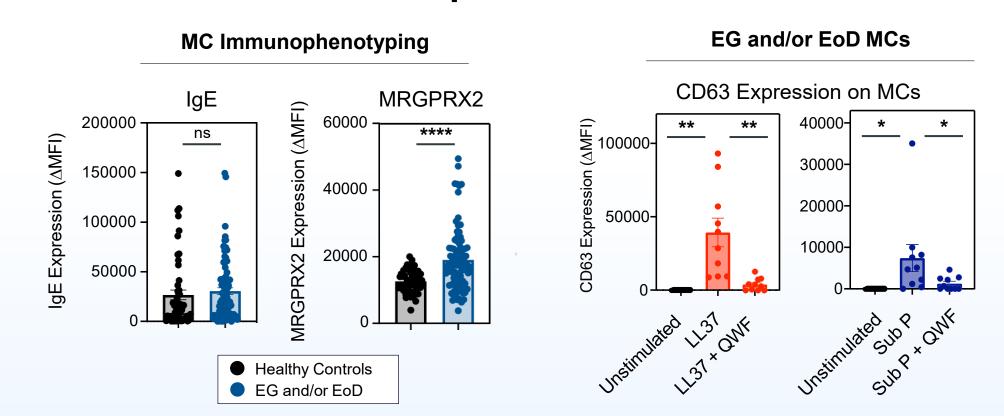
* p <0.05; ** p<0.01; *** p<0.0001; **** p<0.0001

Figure 7. MCs from EG and/or EoD Biopsies are Transcriptionally Activated and Distinct Compared to Non-Diseased GI MCs



 MCs from EG and/or EoD patients are globally activated and display upregulated disease-relevant genes

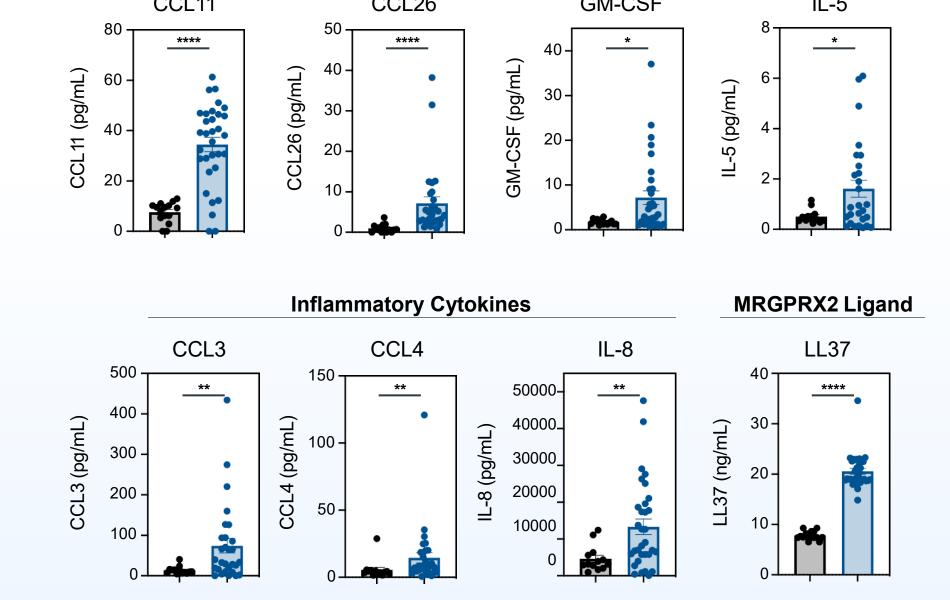
Figure 8. MRGPRX2 is Elevated and Functional on MCs from EG and/or EoD Biopsies



• MRGPRX2, but not IgE expression was elevated on EG and/or EoD MCs and known MRGPRX2 ligands (LL37 and Substance P) induced activation of MCs from EG and/or EoD biopsies that was inhibited by the MRGPRX2-blocking peptide, QWF

*p <0.05; ** p <0.01; *** p <0.0001; **** p <0.0001

Figure 9. Eosinophil Chemokines and Cytokines, and MRGPRX2 Ligands are Locally Elevated in EG and/or EoD Biopsy Supernatants

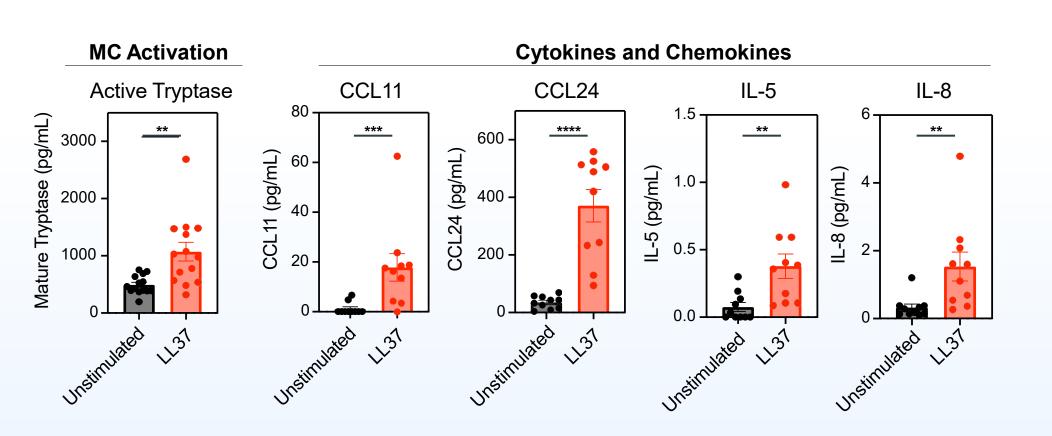


- Biopsies from symptomatic EG and/or EoD patients produced significantly elevated levels of Eos chemokines and inflammatory cytokines, consistent with local tissue inflammation
- In addition, levels of the MRGPRX2 ligand, LL37 were elevated in biopsies from EG and or/EoD patients compared to Healthy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

* p <0.05; ** p<0.01; *** p<0.0001; **** p<0.0001

EG and/or EoD

Figure 10. LL37 Induces MC Activation and Production of Eosinophil and Inflammatory Cytokines in EG and/or EoD Biopsies



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

* p <0.05; ** p<0.01; *** p<0.0001; **** p<0.000

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 globally activated
- Symptomatic EG and/or EoD patients display significant local gastric and duodenal inflammation characterized by elevated levels of Eos chemokines and inflammatory cytokines
- 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