PROJECT

Supervisor:	Ungaro Federica
Title:	Single-cell and spatial dissection of eosinophil-rich ileocolitis: from eosinophilic gastrointestinal disease to inflammatory bowel
Curriculum:	ECM
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/units/experimental-gastroenterology-unit/federica-ungaro.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Eosinophil-rich inflammation of the gastrointestinal tract represents a diagnostic and biological challenge, particularly in the ileocolonic region, where eosinophilic gastrointestinal disorders (EGIDs) and inflammatory bowel disease (IBD) may present with overlapping clinical, endoscopic, and histologic features[1]. Eosinophilic colitis (EoC), a rare form of EGID, is characterized by mucosal eosinophilic infiltration and variable gastrointestinal symptoms, but its natural history and relationship with IBD remain poorly understood [2]. Emerging evidence suggests that EoC may precede, overlap with, or evolve into IBD in a subset of patients, raising the question of whether eosinophilic colitis represents a distinct disease entity or an early eosinophil-predominant phenotype of IBD [2].

Eosinophils are increasingly recognized as key regulators of intestinal immune homeostasis, epithelial barrier integrity, and tissue remodeling, rather than simply effector cells in allergic disease[3]. In IBD, eosinophils are frequently increased in the intestinal mucosa and have been associated with disease activity, fibrosis, and worse clinical outcomes. Conversely, primary eosinophilic gastrointestinal disorders are typically driven by Th2-mediated immune responses characterized by IL-4, IL-5, IL-13, and eotaxin-mediated eosinophil recruitment and activation[4,5]. However, recent transcriptomic studies suggest that eosinophilic colitis may have a distinct molecular profile compared with IBD, although a subset of patients with eosinophilic colitis later develop IBD during follow-up.

Distinguishing primary eosinophilic gastrointestinal disease from early or atypical IBD remains challenging because classic histologic features of IBD, such as architectural distortion, basal plasmacytosis, or granulomas, may be absent in early disease stages, while endoscopic findings may be normal or nonspecific. As a result, some patients are classified as having eosinophil-rich or unclassified ileocolitis, representing a diagnostic grey zone between EGIDs and IBD[2].

Rationale and hypothesis

We hypothesize that eosinophil-rich ileocolitis includes biologically distinct conditions that can be distinguished by specific molecular pathways, cellular states, and spatial tissue organization, and that advanced tissue profiling can identify disease-specific signatures capable of distinguishing primary EGIDs from IBD-associated eosinophilic inflammation and from early IBD.

Objectives and specific aims

1) To retrospectively characterize eosinophil-rich ileocolitis using immunohistochemistry and targeted transcriptomic analysis in FFPE ileocolonic biopsies

2) To identify the cellular subtypes, immune cell states, and molecular pathways characterizing eosinophil-rich ileocolitis across the spectrum from EGIDs to IBD.

3) To define the spatial organization of eosinophil-rich inflammation and identify disease-specific inflammatory niches using spatial transcriptomics and multiplex immunofluorescence.

4) Exploratory Aim: To explore whether molecular and spatial tissue signatures identified in unclassified eosinophil-rich ileocolitis are associated with subsequent diagnosis of IBD during clinical follow-up.

Expected outcomes

This project will provide a comprehensive molecular and spatial characterization of eosinophil-rich ileocolitis and clarify the relationship between primary eosinophilic gastrointestinal disease and inflammatory bowel disease. We expect to define distinct epithelial, stromal, and immune programs underlying primary eosinophilic disease versus eosinophil-rich IBD, enabling a biologically informed classification beyond conventional histology. Single-cell analysis will identify disease-specific cellular states and potential transitional phenotypes in unclassified cases, while spatial transcriptomics will resolve the organization of inflammatory niches and tissue microenvironments.

Overall, the project will advance the understanding of eosinophil-driven inflammation and establish a framework for the molecular classification of eosinophil-rich gastrointestinal disorders.

Skills that the student should acquire (max. 600 characters including spaces):

- Isolation of primary cells from surgical specimens or endoscopic biopsies
- Competences in transcriptomics analysis (single-cell RNA sequencing and Spatial Transcriptomics)
- Competencies in molecular and cellular biology techniques (RNA extraction, reverse-transcription, RT-PCR, cells (co-)culture, plasmid production, and cell lentiviral transduction, cell sorting, cito-fluorimetry, and immunofluorescence analysis)
- Capability to critically discuss results
- Capability to write reports and the final manuscript, along with the final thesis
- Independence in coordinating experiments, under DoS's supervision

References (max. 15)

1. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). *J Allergy Clin Immunol* 2004;**113**(1):11–28; quiz 29. Doi: 10.1016/j.jaci.2003.10.047.
2. Sumiyoshi N., Toyonaga T., Maeda M., Tanaka M., Shimoda M., Saruta M. Challenges in the Diagnosis of Eosinophilic Colitis: Insights from Cases Initially Diagnosed as Inflammatory Bowel Disease. *Inflamm Intest Dis* 2026;**11**(1):110–5. Doi: 10.1159/000550696.
3. Canavese G., Falco EC., Ribaldone DG. Sampling Extension, Chronic Infiltrates, and Eosinophils: Support for the Evaluation of Histological Healing in Inflammatory Bowel Disease with Endoscopic Remission. *Diagnostics (Basel)* 2026;**16**(5). Doi: 10.3390/diagnostics16050739.
4. Bhattacharya B., Carlsten J., Sabo E., Kethu S., Meitner P., Tavares R., et al. Increased expression of eotaxin-3 distinguishes between eosinophilic esophagitis and gastroesophageal reflux disease. *Hum Pathol* 2007;**38**(12):1744–53. Doi: 10.1016/j.humpath.2007.05.008.
5. Straumann A., Bauer M., Fischer B., Blaser K., Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. *J Allergy Clin Immunol* 2001;**108**(6):954–61. Doi: 10.1067/mai.2001.119917.

6. Massimino L., Parigi TL., Riva M., Nicolò S., Errico C., Spanò S., et al. Spatiotemporal analysis of Crohn's disease reveals PECAM2 signaling at the basis of the inflammation-to-fibrosis transition. *J Crohns Colitis* 2025;**19**(8). Doi: 10.1093/ecco-jcc/jjaf130.