Connecting the Infant Gut Microbiome and Allergic Disease via Eosinophil Modulation

Allergic diseases such as food allergies, asthma, and eosinophilic gastrointestinal disorders affect approximately 40% of individuals in industrialized countries, and their incidence continues to rise. These conditions are notoriously difficult to treat and prevent, and their onset typically begins in infancy or early childhood. Eosinophilic inflammation is a hallmark of allergic disease, but eosinophils are increasingly recognized to have broader roles in mucosal homeostasis and immune development. Indeed, studies in germ-free mice demonstrate that the presence of a normal microbiota reduces eosinophil recruitment and survival in the gut, whereas **microbial imbalance can lead to elevated eosinophil levels and heightened allergic sensitization** (Jiménez-Saiz *et al.*, 2020). It is unclear how the infant gut microbiome shapes eosinophil abundance, activation, and function.

The introduction of solid foods alongside breastmilk or formula- known as complementary feeding, typically occurring between 5 and 12 months of age- is a critical window for immune system development and a period during which diet has a profound impact on the gut microbiome. Recent human and animal studies suggest that **the presence of diet-sensitive microbes in early life can influence allergic inflammation**. A large observational study (Hose *et al.*, 2021) found that high meat consumption results in overgrowth of iron-dependent bacteria (e.g. *Acinetobacter*) and confers an increased risk of asthma. Fiber-free diets in mice led to mucus erosion, elevated eosinophils, and enhanced allergic sensitization (Parrish *et al., 2023*). These findings support a model in which early-life diet shapes the microbiome and modulates epithelial immune responses and eosinophil recruitment. However, it remains unclear how specific microbes promoted by different dietary contexts regulate eosinophil development and activation**. This knowledge gap limits our ability to harness the microbiome as a tool to regulate eosinophil activity and prevent downstream allergic disease.**

To address this, we propose to colonize germ-free mice with fecal samples from infants exposed to different complementary diets (vegetable- vs. meat-based) and characterize how the resulting microbiota influence eosinophil dynamics. Our **overall objective** is to determine how human-derived, diet-specific gut microbial communities shape eosinophil levels, activation states, and immunological function. Our **long-term goal** is to identify microbiome-based interventions- such as targeted dietary strategies or probiotic strains- that reduce eosinophilic inflammation and lower food allergy risk.

Aim 1) Determine how diet-sensitive microbes influence eosinophil accumulation & activation in the gut. High-meat diets in infancy promote iron-utilizing and mucin-degrading bacteria that are linked to barrier dysfunction and mucosal inflammation, and high protein diets in adults are linked with increased eosinophil turnover and inhibition of anti-inflammatory, resident eosinophils. We hypothesize that infant microbiota shaped by vegetarian diets will enhance eosinophil residency in the gut and decrease inflammatory signals. In **Subaim 1A**, we will quantify eosinophils and assess activation markers in intestinal tissue using multiparameter flow cytometry, histological analysis, and transcriptomic analysis of sorted eosinophils. This will establish a functional link between early diet-driven microbiome composition and eosinophilic inflammation and elucidate dietary modulation of allergic disease. In **Subaim 1B**, we will perform multiplex cytokine analysis on intestinal tissue to quantify expression of eosinophil-regulating cytokines and chemokines. Identifying the host immune pathways engaged by microbiota will clarify the mechanisms linking microbial exposure to allergic inflammation and prioritize therapeutic targets for microbiome-based prevention strategies.

Aim 2) Identify microbial taxa and metabolic pathways associated with eosinophil regulation and map their intestinal niches. Microbiota-derived metabolites play a critical role in modulating eosinophil homeostasis and allergic inflammation. SCFAs such as butyrate and propionate- produced by Clostridia in the distal gut- exert immunoregulatory effects, whereas mucin-degrading microbes (e.g., *Akkermansia muciniphila*) and iron-dependent taxa (e.g., *Enterobacteriaceae*) can disrupt the mucus barrier and promote eosinophil activation. We hypothesize that SCFA-producing taxa in the ileum and colon suppress eosinophil activation, while mucin-degrading and iron-scavenging microbes in the duodenum and jejunum enhance it. We will address this by strain-resolved metagenomic sequencing of fecal and regionalized small intestinal samples to profile microbial taxa and functions (e.g., SCFA biosynthesis, mucin degradation). We will associate these features with eosinophil abundance and activation across gut regions to identify candidate microbes and pathways for mechanistic follow-up and therapeutic targeting.

By identifying specific microbial and immunological pathways that shape eosinophilic inflammation during infancy, this work will lay the groundwork for the development of targeted interventions, such as probiotics or dietary strategies, to prevent allergic conditions before they emerge.

Abbreviated Research Plan: Five groups of C57BL/6 germ-free mice (n=5/group) will be used. One group will remain germ-free as a control. Two groups will be humanized with stool from one-year-old infants fed a vegetarian diet from 5–12 months of age, and two groups with stool from infants fed a meat-based diet over the same period. Infant fecal samples are on-hand in the Olm lab and were collected as part of an ongoing RCT (ClinicalTrials.gov NCT05012930). Human infant microbiota may colonize differently in germ-free mice but can still reveal key taxa and functions (Luk et al., 2018). Mice will be maintained under gnotobiotic conditions for 28 days before sacrifice and sample collection. All procedures will be conducted at the CU Anschutz Gnotobiotic Core; letter of support available upon request. Deep metagenomic sequencing (15 gigabase-pairs per sample) will be performed on the luminal contents of the duodenum, ileum, jejunum, and colon of each mouse, for a total of 100 generated metagenomes. These will be analyzed using strain-resolved bioinformatic approaches that have been developed or used previously by PI Olm. Intestinal eosinophil profiling in the duodenum, ileum, and jejunum will be achieved by immunofluorescent staining of tissue sections and fluorescence-activated cell sorting (FACS) of digested tissue, which will enable quantification of surface markers indicative of activation status including CD11c, PD-1L, CD22, CD63, and CD69, as well as transcriptomic analysis of sorted eosinophils. Sequencing libraries will be prepared in the Dunn lab, sequenced through the CU Anschutz Genomics Core, and analyzed using pipelines previously used by Dr. Dunn. Multiplex cytokine analysis will be performed on supernatants from intestinal tissue explants using the Meso Scale Discovery platform instrumentation available through the Mucosal Inflammation Program on the Anschutz campus.

Expected Outcomes and Impact: The data generated in these aims will result in at least one primary research article, and the datasets generated through the microbiome analysis and transcriptional profiling of GI eosinophils will support future hypotheses, publications, and projects. The study will generate a bank of paraffin-embedded tissue blocks from the small intestine that may be probed in the future using immunofluorescence or spatial transcriptomics. At the time of sacrifice, in parallel with the intestinal tissue collection, we will also collect, stain, and sort eosinophils and type 2 innate lymphoid cells from lung as well as eosinophil progenitor populations from the bone marrow. Future studies may therefore draw on these samples to probe the effect of early microbial colonization on the gut-lung axis and innate immune training, both of which are active areas of research focus by federal and foundational organizations. Overall, the translational potential of this work is high, with clear applications to clinical trial design, infant feeding guidelines, and therapeutic development.

Investigator Qualifications and Interdisciplinarity: This project brings together two early-career investigators with complementary expertise in microbiome science and eosinophil biology, enabling an interdisciplinary approach to a complex problem in early-life immune development. Dr. Matthew Olm is a tenure-track Assistant Professor at CU Boulder with extensive experience in metagenomics, human infant studies, and bioinformatic method development. Dr. Julia Dunn is a tenure-track Assistant Professor at CU Anschutz, whose work combines *in vivo* and *ex vivo* models to dissect eosinophil-epithelial interactions and immune regulation in allergic inflammation. The Dunn Lab has a current IACUC protocol addressing the role of eosinophils in inflammation (#01377) and the expertise to collect all samples described in this proposal. Moreover, Dr. Dunn's affiliation with the Department of Pediatrics and the Gastrointestinal Eosinophilic Diseases Program is ideal for facilitating future clinical studies on the role of early life microbial colonization in the onset of allergic disease, which will leverage Dr. Olm's expertise in microbial bioinformatics. This collaboration is anchored by institutional support, access to gnotobiotic and immunology facilities, and a shared vision for the potential of microbiome-based interventions to prevent allergic disease. Drs. Olm and Dunn have not collaborated previously, and this project aims to seed a long-term, interdisciplinary research program in microbiome-immune interactions.

Extramural Sustainability: This pilot study generates preliminary data for a larger research program aimed at understanding how early-life microbial exposures modulate eosinophil function and influence allergy risk. The ample preliminary data and publication that are anticipated to result from this project will support an R01 to the NIH (NIDDK) after completion of this pilot award to expand this line of inquiry into additional animal models and human infant cohorts, with a focus on linking diet-driven microbiota changes to eosinophilic inflammation and immune development. Second, we will pursue a USDA AFRI A1343 grant (Food and Human Health) to develop microbiome-informed complementary feeding strategies that prevent allergic disease. We have confirmed the applicability of this research area with Dr. Kristopher Grimes (National Program Leader, USDA). Finally, we will track emerging funding opportunities from specialized organizations like the American Partnership for Eosinophilic Disorders (APFED; which awarded $100K HOPE Pilot Grants in 2022 and 2024) and the Food Allergy Fund (which awarded $500K Microbiome Challenge in 2022).