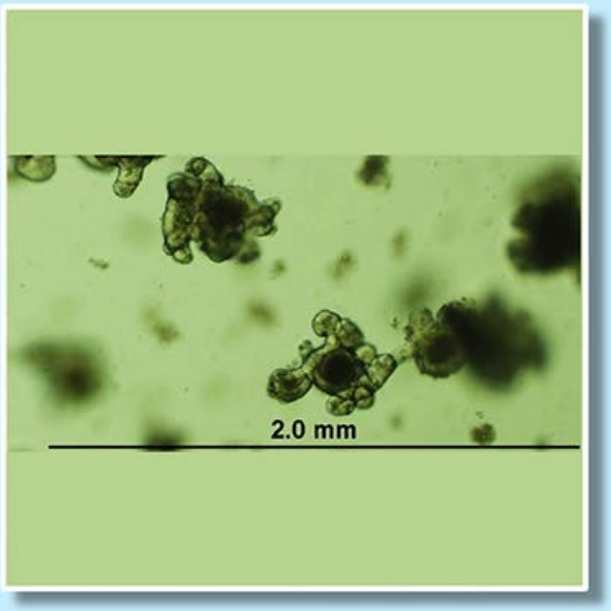
SMRITHI VALSARAJ

Faculty Research Mentor: Dr. Ajay Gulati
Department of Pathology



Enteroid 'Mini Guts' Can be Used to Study Paneth Cell Function

Commensal Gut Bacteria Regulate Paneth Cell Function Putatively Via Signaling Through the Sub-epithelial Tissue

Crohn's Disease (CD) is an inflammatory disorder of the intestines caused by a dysregulated immune response to resident gut bacteria. Presently, most treatments for CD suppress the host immune system, leading to adverse side effects such as increased susceptibility to infections. Hence, low-risk alternative treatment options are needed. Studies suggest that defects in Paneth cells (PCs) contribute to CD pathogenesis. PCs are specialized epithelial cells that regulate the intestinal microbiota through the secretion of antimicrobial peptides (AMPs). However, the reciprocal impact of the gut microbiota, as well as the role of the underlying intestinal stroma on PC function is still unclear. We hypothesized that microbes induce PC AMP expression through signaling from sub-epithelial tissue of the intestines. To test this hypothesis, we compared the abundance of selected PC AMP mRNAs between specific pathogen free (SPF) mice that have a normal gut microbiota, and germ-free (GF) mice that have a sterile gut. AMP transcript levels were also compared in an *in vitro* system (enteroids) cultured from SPF and GF epithelial cells. The enteroid system is devoid of underlying stroma and bacteria, and thus provides a model to study the impact of these components on PC function. *In vivo*, AMP expression was significantly higher in SPF small intestine tissue than in GF tissue. However, *in vitro*, AMP expression in SPF enteroids decreased to that of GF enteroids over time. Interestingly, with the addition of sub-epithelial tissue to the enteroid system, AMP expression in SPF enteroids was restored to our *in vivo* measurements. The addition of bacterial products (such as LPS) without sub-epithelial tissue did not induce AMP expression. Based on these findings, we conclude that commensal microbiota regulate AMP expression of the PCs, putatively through signaling from the sub-epithelial tissue. This work also highlights the limitations of the enteroid system when studying PC function since the sterile culture conditions neglect possible bacterial interactions with the underlying stroma. Hence, this limitation should be taken into consideration when using enteroids for future PC studies in CD.