Group 2 Innate Lymphoid Cells Do Not Release Amphiregulin in Culture

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Introduction: Group 2 Innate Lymphoid Cells (ILC2s) may be involved in a protective immune response in inflammatory bowel disease (IBD). ILC2s are shown to induce goblet cell differentiation via IL-13. ILC2s may also modulate intestinal epithelial growth and/or survival through release of amphiregulin (AREG), a ligand of the epidermal growth factor receptor. In IBD mouse models, it is thought that IL-33, a stimulatory cytokine to ILC2s, induces epithelial cell growth and repair in an AREG-dependent manner.

Hypothesis: In patient-derived colonoids, ILC2-derived AREG will enhance the survival and proliferation of intestinal epithelial cells.

Methods: Pediatric patients ages 4-21 undergoing colonoscopy for clinical indications were our source for up to 16 mL of peripheral blood and 8 rectosigmoid colon biopsies. PBMCs (Peripheral Blood Mononuclear Cells) were enriched for ILC2s using negative magnetic selection and stimulated with cytokines to expand ILC2s (IL-2, IL-7, and IL-33) or other ILC2-enhancing cytokines to induce AREG. Crypts from biopsies were incubated with EDTA and scraped to separate epithelial cells from the lamina propria. Crypt cells were grown and differentiated as colonoids in growth media in a Matrigel matrix. ILC2s and colonoids were cultured separately and together. Supernatants were collected for ELISA and cells were lysed to isolate RNA for qRT-PCR.

Results: ILC2s cultured alone did not release ELISA-detectable amounts of AREG. When compared to ILC2s alone, ILC2s cultured with colonoids had higher levels of AREG expression but lower expression than colonoids. PBMCs contained higher levels of AREG expression, and flow cytometry showed that most AREG producing cells were CD3⁺ (T-cells). Even though there were more AREG producing T-cells by number, a greater proportion of CD3⁻CD161⁺ (ILC2s) produced AREG, suggesting that a subset of ILC2s do make AREG, but our culture conditions did not induce them to release it.

Conclusions: Although ILC2s express AREG and a subset synthesize AREG, we could not detect AREG in supernatants from cultured ILC2s. Further, we saw evidence that colon epithelial cells express and release AREG. Future directions include isolating tissue-resident ILC2s and using flow cytometry to look for phenotypic differences between AREG-producing and non-AREG producing variants.

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