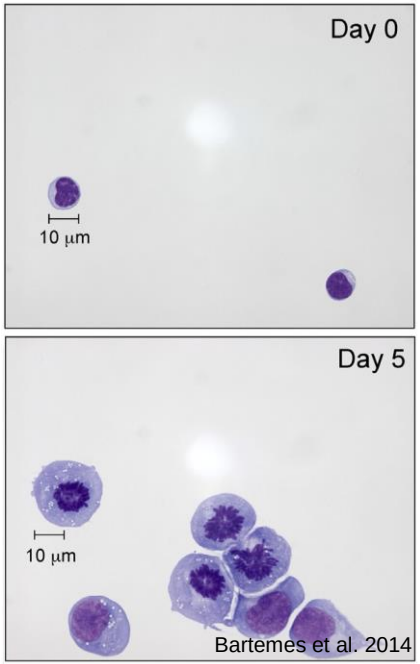
# ILC2 Cells in the Holsapple Laboratory

2016 CRIS Annual Meeting

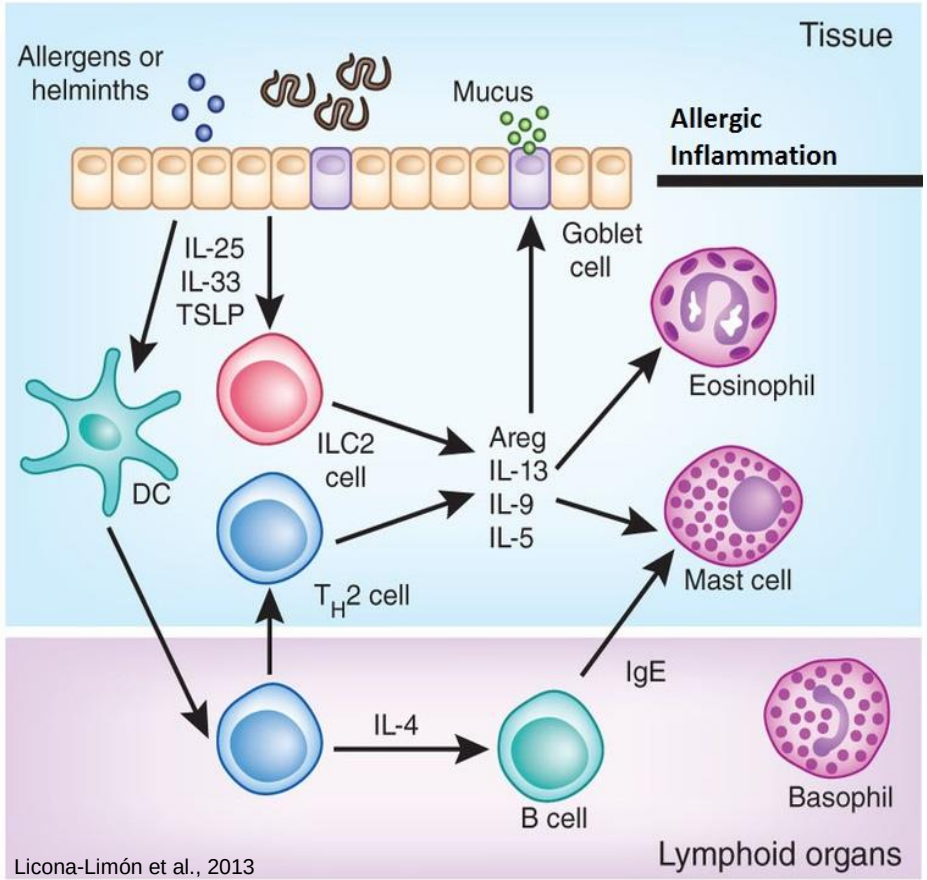
# ILC2 Characteristics in Humans

- Non-cytotoxic, classic lymphoid morphology but lacking lineage markers
- Primarily found in gut, lung, and inflamed nasal polyps
  - Also found less abundantly in peripheral blood
- Landmark papers identify $CD127^+$, $CD161^+$, $CRTH2^+$
- Activated by IL-25, IL-33
- Produce IL-5, IL-13
- Characteristic intranuclear expression of GATA3[5]



# Type 2 Responses

- Allergens and helminths disrupt gut or airway epithelium, releasing alarmins IL-25, IL-33
- Activated ILC2s release type 2 cytokines, induce $T_H2$ response
- IL-13 induces mucus secretion, IL-5 recruit eosinophils and mast cells




Licona-Limón et al., 2013

# Importantly from Human Studies:

- ILC2s from PBMCs produce large quantities of IL-5 and IL-13 when stimulated with IL-2 and IL-25, IL-33 *in vitro*[1,3,5,6]
- Type-2 responses are enhanced and ILC2s increased in patients with allergic asthma (AA)[1], atopic dermatitis[8], and allergic rhinitis (AR)[3]
- Direct correlation between allergen exposure and increased ILC2s in patients with AA[1], AR[2]
- Freshly isolated ILC2s from PBMCs[4,5,8], skin[8], gut[4,5] and airway epilethelia[5] display similar cytokine profiles and maintain those profiles after expansion *in vitro*[5]

# CONTEXT – RELEVANCE TO CRIS MISSION

- CRIS to offer unbiased and transparent evaluation of new technology that can be applied to evaluate ingredient safety
- Although rare, food coloring agents can cause allergic reactions – sometimes severe
- Food companies are voluntarily moving away from using artificial colors – because of consumer pressure about health concerns, including allergenicity

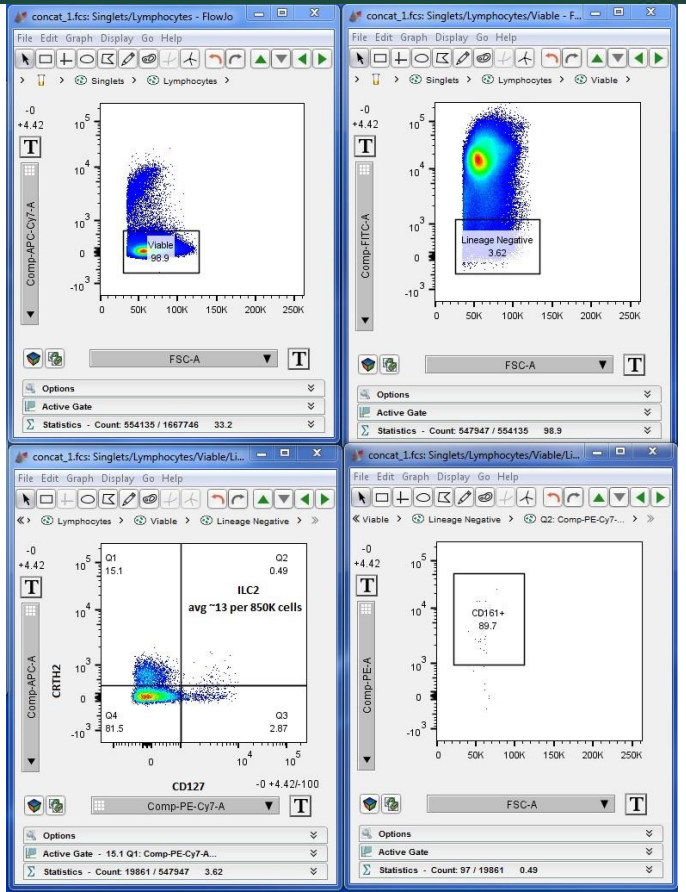
# Studying ILC2s in the Holsapple Lab:

Our goal is to describe how agents alter ILC2 function in the pathophysiology of type 2 inflammatory diseases. Possible projects include:

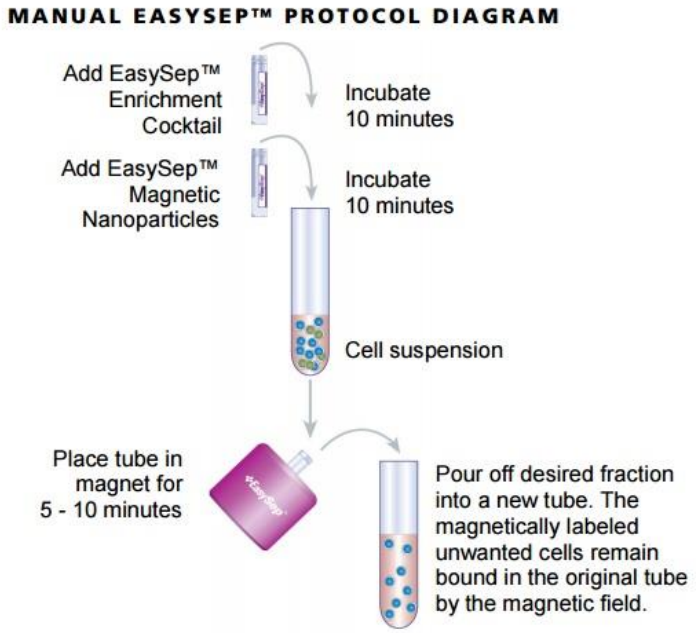
- An *in vitro* model examining ILC2s as mediators with potential sensitizers and food allergens in inflammatory reponses
- Re-examining food allergy studies done prior to the discovery of ILC2s
- Modulating cytokine profiles and molecular pathways as potential therapeutic targets

# Initial Identification

- Isolated PBMCs from healthy donors
- Surface-stained for Lineage markers, CD127, CRTH2, and CD161
- Identified approxiametly 0.01% of PBMCs as ILC2s by flow cytometry
- Literature also reports 0.01% of PBMCs[1,3,5]



# Immunomagnetic Depletion, Sorting




- Use of immunomagnetic separation to deplete Lineage$^+$ cells has several benefits
  - Depletes Lin$^+$ cells for faster sorting
  - Generates PBMCs for feeder system
  - Generates enhanced fractions for co-culture experiments
- Initial experiments show modest enhancement of ILC2 cells, extrapolates to 4 hours sorting time and 20,000 ILC2 cells recovered

# Currently Working on Sorting and Cell Culture

- ILC2s can be expanded *in vitro*, up to 5000 fold increase[4]
  - Our 20,000 ILC2s can become 100 million within a month
- I have been monitoring the health of sub-lethal gamma irradiated PBMCs as feeder cells
- Post-sort viability is a major concern, we will work on optimizing sheath pressure and sheath fluid with MSU Flow Cytometry Core

# Works Cited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