**Methods**

**Introduction**

- In mice, CD1d+CD5+ (B10) B cells have regulatory properties associated with IL10 production in vitro.
- In humans, we previously found that this phenotype is more frequent in young children than adults.
- Infants show better heart transplant outcomes than older recipients, including acceptance of ABO-incompatible grafts.

**Hypothesizing that these cells contribute to the better graft acceptance in infants, we aimed to determine whether human CD1d+CD5+ B cells are functionally similar to B10 cells in mice.**

**Results**

**Assay 1: IL10 quantification**

- CD1d+CD5+ B cells were cultured parallel to non-CD1d+CD5+ B cells using T-dependent (CD40L+IgM) and T-independent (CD28 T cell, αα) assessed using B cell stimuli plus CD3- supernatants of CD1d+CD5+ B cells cultured for days 2-4
- Supernatants were collected on days 2 to 4 and IL10 concentrations were quantified using an ELISA
- More data across the age spectrum are required to confirm findings and identify additional regulatory B cell phenotypes.

**Assay 2: CFSE proliferation**

- Proliferation of CFSE-stained splenocytes was assessed using B cell stimuli plus CD3-CD28 T cell stimuli, without CD1d+CD5+ B cells and with increasing CD1d+CD5+ B cell proportion at the naturally occurring and the 2, 3, and 5-fold the original proportion
- Effects of CD1d+CD5+ on lymphocyte proliferation were similar but less pronounced in pediatric compared to adult samples.
- In absence (compared to normal quantity) of CD1d+CD5+ B cells, the remaining B cells showed stronger proliferation with both T-dependent and T-independent stimulation, peaking on day 4.
- B10 and non-B10 B cells produced similar IL10 levels with both T-dependent and T-independent stimulation, peaking on day 4.
- In absence (compared to normal quantity) of CD1d+CD5+ B cells, the remaining B cells showed stronger proliferation with both T-dependent and T-independent stimulation (3.4±3.9 fold and 2.7±2.3 fold respectively), while proliferation was reduced with 2X and 3X these cells.
- T cell proliferation was increased by 1.7±1.1 fold in absence of CD1d+CD5+ and slightly decreased with 3X these cells. At 5X the natural proportion of these cells, the large overrepresentation resulted in enhanced T cell proliferation.
- Effects of CD1d+CD5+ on lymphocyte proliferation were similar but less pronounced in pediatric compared to adult samples.

**Discussion**

**Acknowledgements**

- West Lab Members: Catherine Ewen, PhD
- FACS sorting: Catherine Ewen, PhD

- The high prevalence of CD1d+CD5+ B cells in early childhood likely contributes to better graft acceptance.
- More data across the age spectrum are required to confirm findings and identify additional regulatory B cell phenotypes.