Impact of CD1d+CD5+ B Cells on T-dependent and T-independent immune responses in early childhood

Ying Ling[1,3], Esmé Dijke, PhD[1], Lori J West, MD, DPhil, FRCPC[1,2,3] and Simon Urschel, MD[1,3]

[1]Pediatrics, University of Alberta; [2]Surgery, University of Alberta; [3]Medical Microbiology and Immunology, University of Alberta

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.**

Methods

• Human splenocytes were stained with an intracellular dye, Carboxyfluorescein-Succinimidyl-Ester (CFSE), which divides evenly between the daughter cells following proliferation of a parent cell, allowing for the analysis of cell cycles.

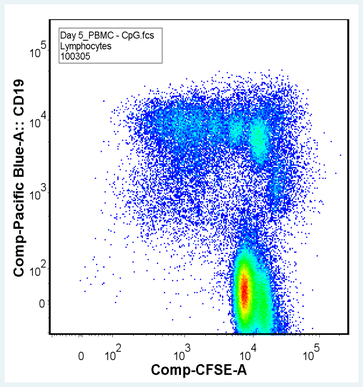


Figure 1. Proliferation of adult human lymphocytes from PBMC when stimulated with CpG (3µg/ml)

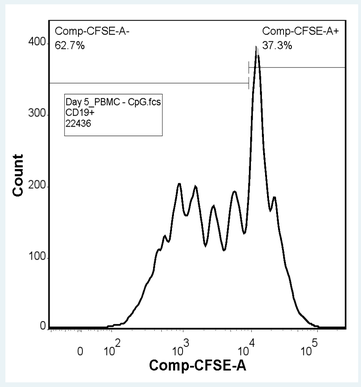


Figure 2. Proliferation of adult human PBMC CD19+ lymphocytes when stimulated with CpG (3µg/ml)

• Using flow activated cell sorting (FACS), CD1d+CD5+ B cells were then sorted from whole splenocytes.

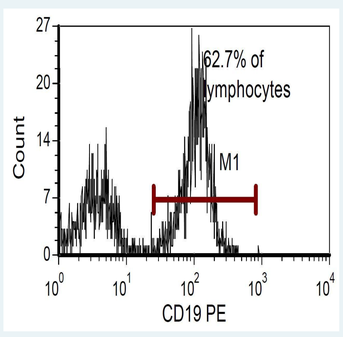


Figure 3. Gate for CD19+ lymphocyte sorting

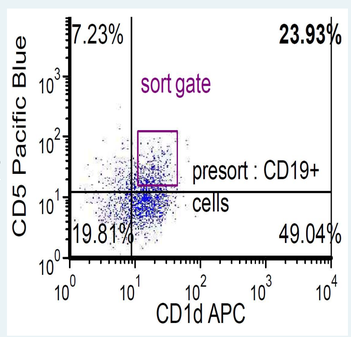


Figure 4. Gate for CD1d+CD5+ B-cell sorting

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 (CpG, IgM) B cell stimuli.
- Supernatants were collected on days 2 to 4 and IL10 concentrations were quantified using an ELISA
- Flow cytometry was used for assessment of proliferation via CFSE staining

Assay 2: CFSE proliferation

- Proliferation of CFSE-stained splenocytes was assessed using B cell stimuli plus αCD3-αCD28 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

Results

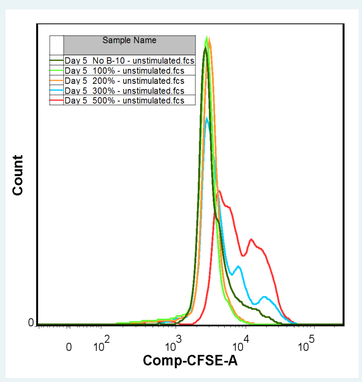


Figure 5. Proliferation of CD19+ 4.4 month pediatric splenocytes after 5 days without stimulation

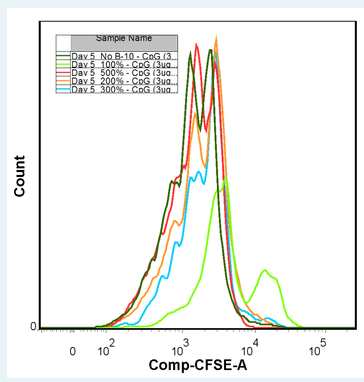


Figure 6. Proliferation of CD19+ 4.4 month pediatric splenocytes after 5 days of T-independent B cell stimulation with CpG (3µg/ml)

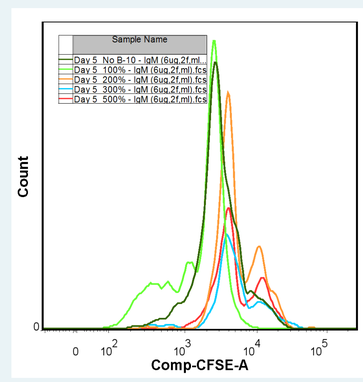


Figure 7. Proliferation of CD19+ 4.4 month pediatric splenocytes after 5 days of T-independent B cell stimulation with IgM (6µg/ml)

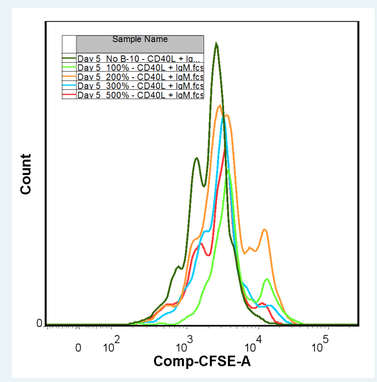


Figure 8. Proliferation of CD19+ 4.4 month pediatric splenocytes after 5 days of T-dependent B cell stimulation with CD40L (1µg/ml) + IgM (6µg/ml)

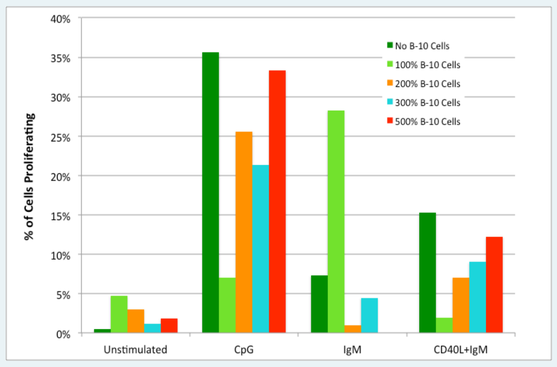


Figure 9. Percent of CD19+ cells proliferated in day 5 culture of 4.4 month pediatric splenocytes. Gating on CFSE was set as shown in figure 2, with the CFSE- gate indicating percent of proliferation

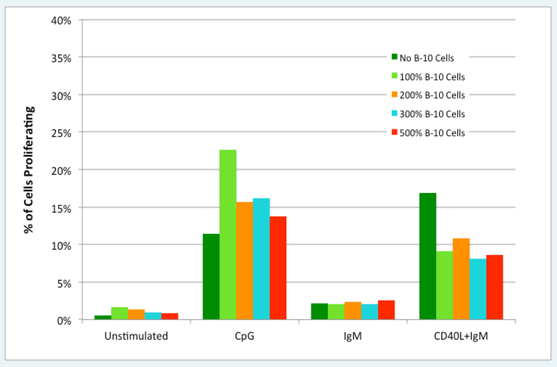


Figure 10. Percent of CD19+ cells proliferated in day 5 culture of 19.4 month pediatric splenocytes. Gating on CFSE was set as shown in figure 2, with the CFSE- gate indicating percent of proliferation

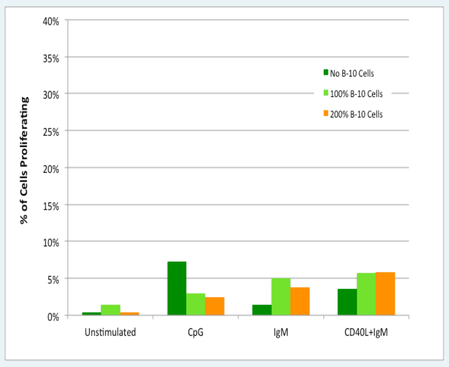


Figure 11. Percent of CD19+cells proliferated in day 5 culture of adult splenocytes. Gating on CFSE was set as shown in figure 2, with the CFSE- gate indicating percent of proliferation

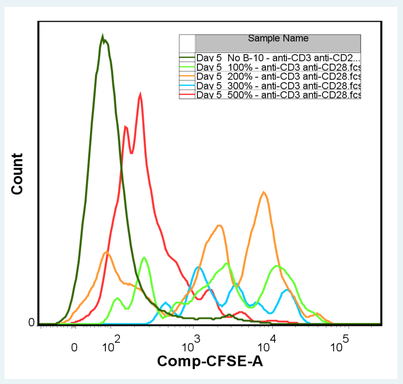


Figure 12. Proliferation of CD3+ 4.4month pediatric splenocytes after 5 days of T cell stimulation with α-CD3 (0.5µg/ml) + α-CD28 (0.5µg/ml)

Figure 13. Percent of CD3+ cells proliferated in day 5 cultures of 4.4 month pediatric, 19.4 month pediatric, and adult splenocytes. Gating on CFSE was set as shown in figure 2, with the CFSE- gate indicating percent of proliferation

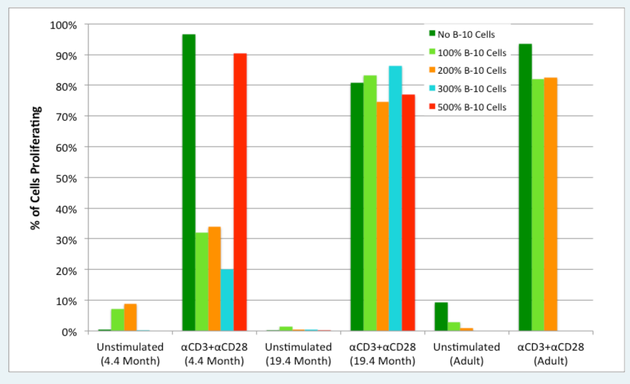
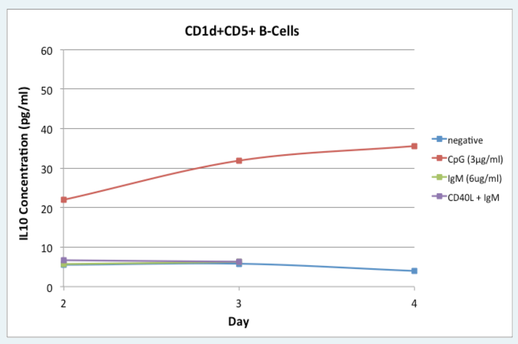


Figure 14. IL10 concentrations in the supernatants of CD1d+CD5+ B cells cultured for days 2-4



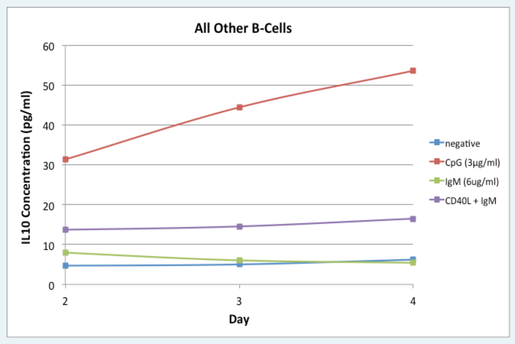


Figure 15. IL10 concentrations in the supernatants of residual B cells cultured for days 2-4

Discussion

• 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, this 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.

• **These results indicate that CD1d+CD5+ B cells in humans have regulatory effects on both B cells and T cells, mediated through IL10. However, IL10 was also secreted by other B cell phenotypes suggesting presence of additional regulatory B cells in humans.**

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.

Acknowledgements

• *West Lab Members*
• *FACS sorting:* Catherine Ewen, PhD