Regulatory B Cells in Humans: Identifying the Regulatory Capacity and Interleukin-10 Production of Regulatory B Cell Phenotypes

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Introduction

- In mice, CD1d+CD5+ B cells have regulatory properties associated with interleukin-10 (IL-10) production
- In humans, this phenotype is up to 10 times more frequent in infants than in adults.
- Infants show better heart transplant outcomes than adults, including acceptance of ABO-incompatible grafts.
- However, they also show increased severity of infections with polysaccharide-encapsulated bacteria.
- **We hypothesize that CD1d+CD5+ B cells play a role in the altered immune response during infancy, particularly towards polysaccharides including ABO-antigens and bacteria capsules.**

Methods

Assay 1: IL-10 Quantification

• Using flow activated cell sorting (FACS), CD1d+CD5+ B cells were sorted from non-CD1d+CD5+ B cells.

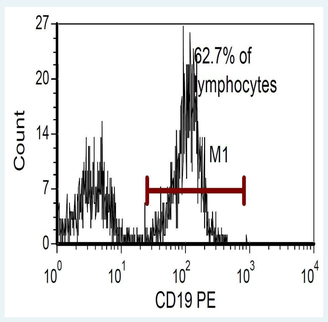


Figure 1. Gate for CD19+ lymphocyte sorting

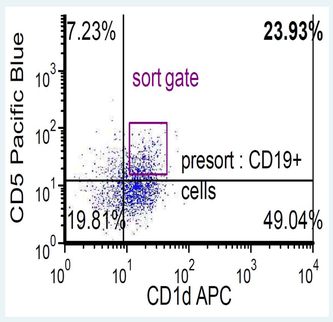


Figure 2. Gate for CD1d+CD5+ B-cell sorting

• CD1d+CD5+ B cells were cultured parallel to non-CD1d +CD5+ B cells using two B cell stimuli:
  - T-independent: CpG (3µg/ml)
  - T-dependent: α-IgM+CD40L (6µg/ml+1µg/ml)
• Supernatants were collected on day 4 and IL-10 concentrations were quantified using an ELISA

Assay 2: CFSE Proliferation

• Human lymphocytes were stained with an intracellular dye, Carboxyfluorescein-Succinimidyl-Ester (CFSE),

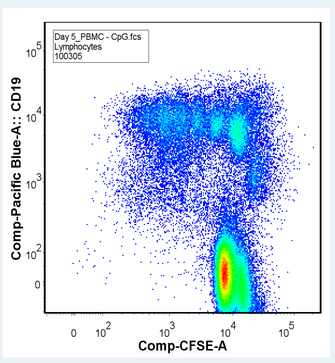


Figure 3. PBMC when stimulated with CpG (3µg/ml)

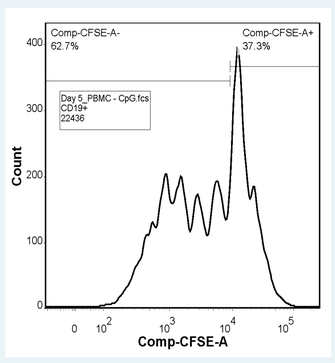


Figure 4. CD19+ PBMC when stimulated with CpG (3µg/ml)

• Cultured 3 conditions of peripheral blood mononuclear cells (PBMC)
  - PBMC^{depleted} – CD1d+CD5+ B cells removed
  - PBMC^{original}
  - PBMC^{double} – CD1d+CD5+ B cells at 2x proportion
• Cultured 6 days with following stimulation:
  - CpG, α-IgM+CD40L
  - α-IgM+CrossLinker (6µg/ml+10µg/ml)
  - α-CD3+α-CD28 (0.5µg/ml+0.5µg/ml)

For all assays, investigator was blinded to age of sample

Results

IL-10 Quantification

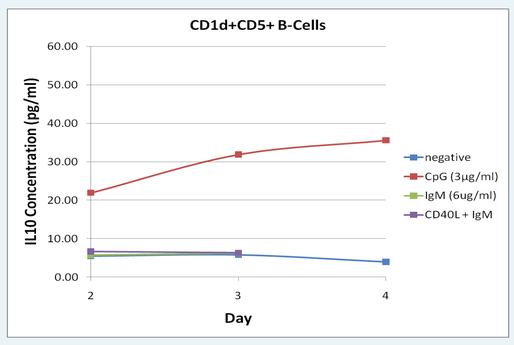


Figure 5. IL-10 concentrations in the supernatants of CD1d+CD5+ B cells cultured for days 2-4

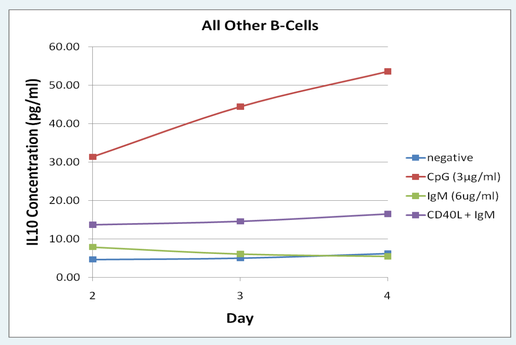


Figure 6. IL-10 concentrations in the supernatants of non-CD1d+CD5+ B cells cultured for days 2-4

	CD1d+CD5+ B Cells	Non-CD1d+CD5+ B Cells
Unstimulated	N/D	N/D
CpG	42.1	77.1
IgM+CD40L	25.7	N/D

Table 1. IL-10 concentrations (pg/ml) in the supernatants after culturing for 4 days

*N/D = not determinable; concentration was lower than what was determined in standards (OD450 < 0.0775)

CFSE Proliferation (Example – UA-672)

Figure 7. B cell proliferation following 6 days of T-independent stimulation with CpG (3µg/ml)

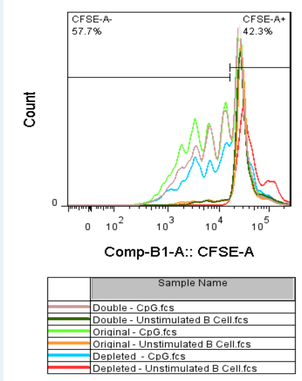


Figure 8. B cell proliferation following 6 days of T-dependent stimulation with α-IgM + CD40L (6µg/ml + 1µg/ml)

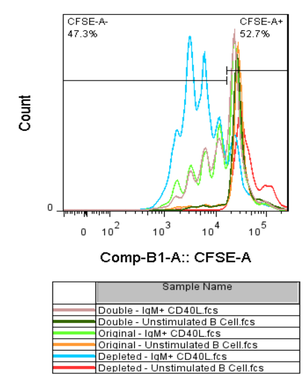
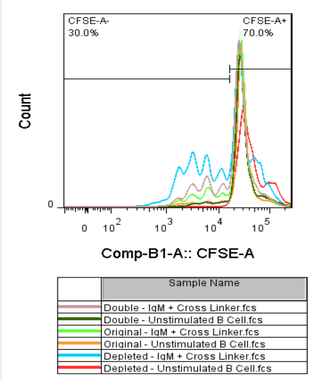


Figure 9. B cell proliferation following 6 days of T-independent stimulation with α-IgM+CrossLinker (6µg/ml + 10µg/ml)



Complete Proliferation Results

Boxplots illustrate 25th percentile, median, and 75th percentile. Mean shown in dotted line.

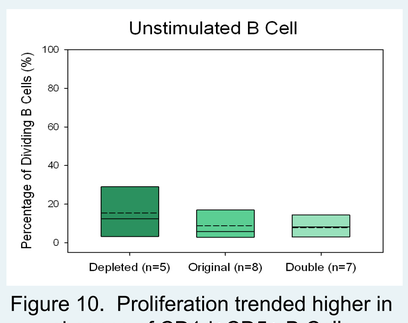


Figure 10. Proliferation trended higher in absence of CD1d+CD5+ B Cells

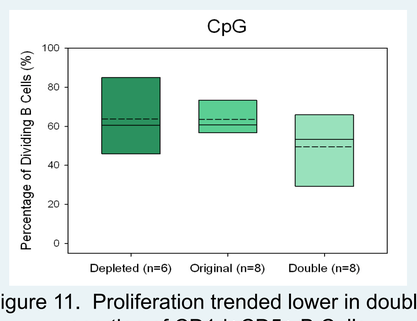


Figure 11. Proliferation trended lower in double proportion of CD1d+CD5+ B Cells

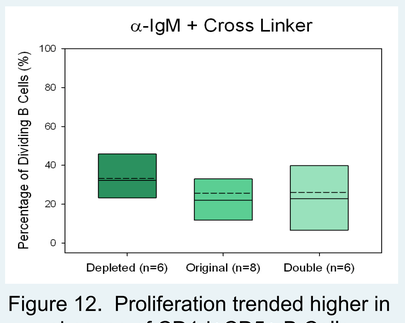


Figure 12. Proliferation trended higher in absence of CD1d+CD5+ B Cells

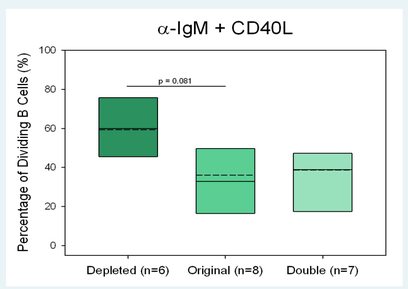


Figure 13. Proliferation trended higher in absence of CD1d+CD5+ B Cells

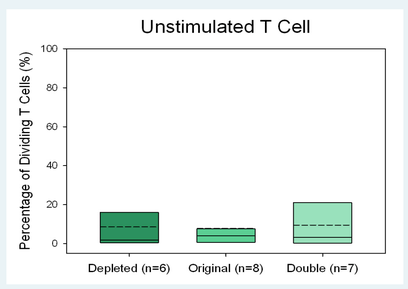


Figure 14. Proliferation similar in all conditions of CD1d+CD5+ B Cells

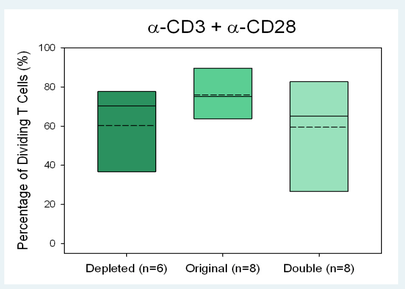


Figure 15. Proliferation trended higher in presence of CD1d+CD5+ B Cells

Conclusion

- When stimulated with α-IgM+CD40L, IL-10 levels were seen in CD1d+CD5+ B cells but not in non-CD1d+CD5+ B cells
- However, presence of IL-10 was also seen in non-CD1d+CD5+ B cells when stimulated with CpG
- When stimulated with α-IgM+CD40L, frequency of proliferated B cells was 27% higher in absence of CD1d+CD5+ B cells compared to the original proportion (P=0.081)
- A similar trend was seen in all B cell stimulation conditions
- In contrast, both absence and double proportion of CD1d+CD5+ B cells had little effect on T cell proliferation

- **These results indicate that CD1d+CD5+ B cells in humans may inhibit the proliferation of B cells**
- **Evidence of IL-10 production in non-CD1d+CD5+ B cells suggests the existence of further phenotypes of regulatory B cells in humans**

- Analyses of further phenotypes of IL-10 producing cells and age related differences are underway

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