ANALYSING DEVELOPMENTAL IMMATURITY IN B CELL INTRACELLULAR SIGNALLING

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BACKGROUND

- Immune responses to T-independent (TI) antigens, such as polysaccharides, are impaired during early childhood
- Infants are more susceptible to invasive infection with encapsulated bacteria
- Infants can accept blood-type (ABO) incompatible heart transplants
- $CD21^{high}$ B cells are crucial in the response to polysaccharides
- CD21 is the receptor for complement fragment C3d
- Co-ligation of the B cell receptor (BCR) and CD21 enhances intracellular signaling (**Figure 1**)

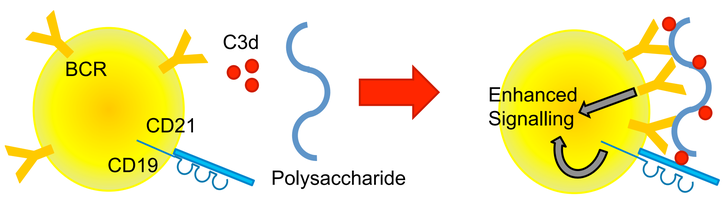


Figure 1. CD21 enhances B cell receptor (BCR) signaling. C3d-coated foreign polysaccharides co-ligate CD21 during engagement of the BCR which lowers the concentration of antigen needed to induce B-cell activation

- We characterised the phosphorylation of Syk and Akt following stimulation of adult or infant B cells (**Figure 2**)
- **We hypothesise that B cell receptor and CD21 co-stimulation will not induce similar intracellular signalling in infant B cells**

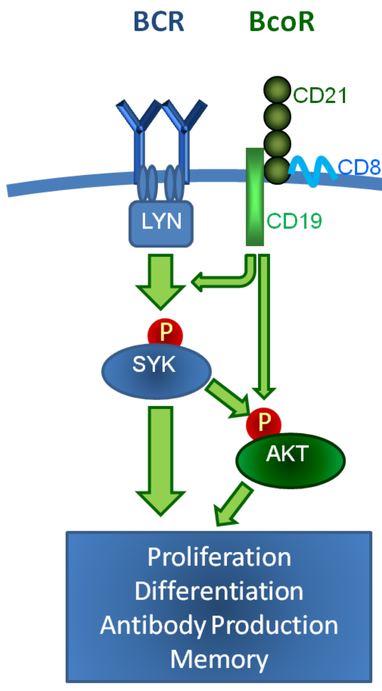


Figure 2. B cell receptor (BCR) intracellular signaling. BCR signalling induces Syk and Akt phosphorylation. Co-ligation of the B cell co-receptor complex (CD19-CD21-CD81) enhances phosphorylation.

MATERIALS AND METHODS

Thawing, Separation, and Equilibration of Cells

- Used human adult or infant splenocytes
- Enrich B cells by negative selection
- Rest at 37°C for 12hrs prior to stimulation

Stimulation of B Cell Receptor Signaling

- Stimulation performed at 37°C
- 10µg/mL $F(ab')_2$ goat anti-human IgM & donkey anti-goat IgG cross-link
- Additional stimulation with 5µg/mL purified C3d
- Cells were fixed at each time point

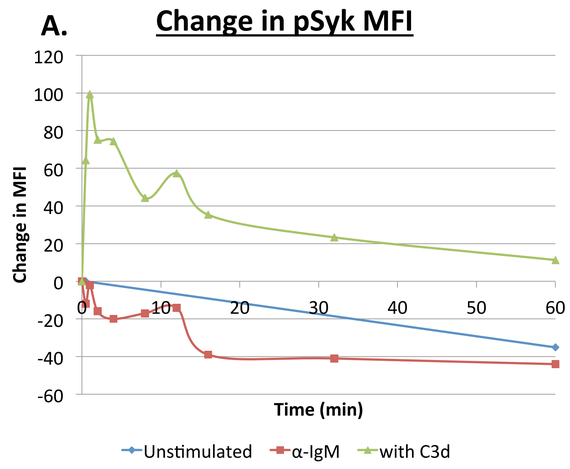
Intracellular Phosphospecific Flow Cytometry

- Cells were permeabilized and stained with phosphorylation-specific fluorescent antibodies: AF488 phospho-Akt(S473) & PE phospho-Syk(Y348)
- Analysis: Miltenyi MACSQuant™ flow cytometer

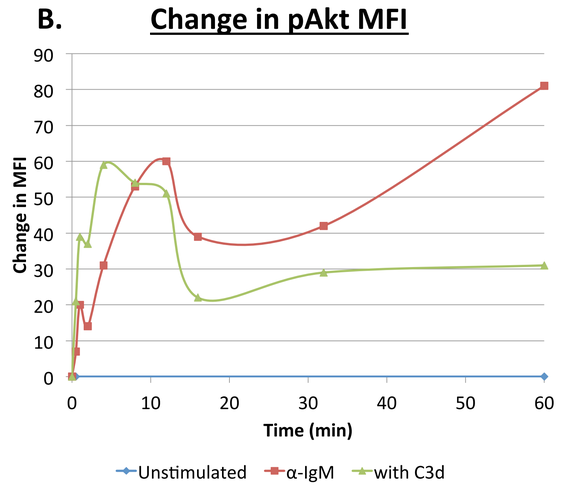
RESULTS

Adult B cells:

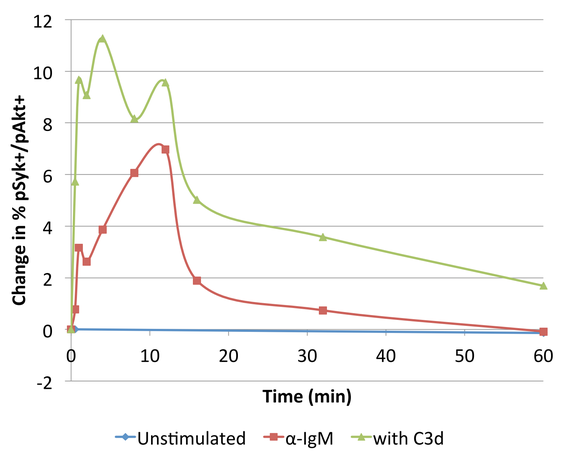
- CD21 co-stimulation induced more phosphorylation of Syk (**Figure 3A**) and an earlier peak in pAkt (**Figure 3B**) compared to BCR stimulation alone
- CD21 co-stimulation induced an earlier and higher peak in the percentage of cells with both signalling proteins phosphorylated compared to BCR stimulation alone (**Figure 3C**)



Cells with C3d co-stimulation had a greater increase in Syk phosphorylation and had an earlier maximum peak (1min) than cells stimulated with anti-IgM BCR stimulation alone

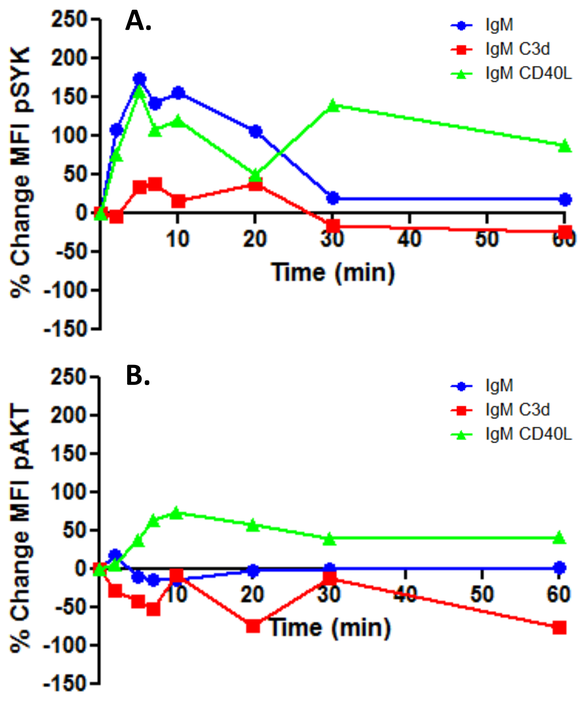


Phosphorylation of Akt occurs sooner (4min) in cells co-stimulated with C3d, compared cells with anti-IgM BCR stimulation alone (12min)



A higher percentage (11.3%) of CD21 co-stimulated cells had phosphorylation of both Syk and Akt at 4min in, compared to 7.0% at 12min in cells with BCR stimulation alone

Figure 3. Intracellular phosphorylation kinetics after stimulation of adult B cells. Change in mean fluorescent intensity (MFI) of phosphorylated Syk (**A**) and Akt (**B**) and percent change in pSyk/pAkt double positive cells (**C**) following anti-IgM stimulation (red), anti-IgM/C3d co-stimulation (green) or no stimulation (blue) in B cells isolated from an adult human spleen



Infant B cells:

- BCR stimulation induced pSyk without progression to Akt phosphorylation
- Addition of CD21 co-stimulation failed to induce either Syk or Akt phosphorylation
- Addition of CD40L co-stimulation induced both pSyk and pAkt (**Figure 4**)

Figure 4. Intracellular phosphorylation kinetics after stimulation of infant B cells. Percent change in mean fluorescent intensity (MFI) of phosphorylated Syk (A) and Akt (B) after anti-IgM (blue), anti-IgM/C3d (red) or anti-IgM/CD40L (green) stimulation of B cells isolated from an infant spleen

DISCUSSION

- **In early childhood, B cell co-receptor stimulation may induce different intracellular signalling compared to later in life**
- Despite similar Syk activation in adult and infant B cells, we did not see persisting Akt activation with T-independent stimulation of infant B cells
- This deficiency may contribute to impaired B cell activation after interaction with T-independent antigens
- This may facilitate persistent acceptance of the donor blood group antigens following blood type (ABO) incompatible heart transplantation
- Further investigation of CD21 co-receptor intracellular signaling in B cells from infants is currently being performed in larger sample sizes
- The B cell signaling pathway may be a future therapeutic target to translate the benefits of the infant immune system to older patients