Intracellular Signaling Cascade in B-Cells in Response to B-Cell Receptor Stimulation with and without Complement Stimulation of the Co-Receptor

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ABSTRACT

- *Introduction*: Within the first two years of life, the immune system has an impaired ability to respond to T-cell independent antigens, including blood-group polysaccharides. This likely contributes to persistent, donor-specific immune tolerance of blood group incompatible heart transplants in early childhood; however, the mechanism is unknown. We investigated the interaction between complement and the T-cell independent B-cell response in an adult immune system.
- *Methods*: Adult peripheral blood mononuclear lymphocytes were stimulated with anti-human IgM antibodies, with or without the addition of C3d. The cells were fixed, permeabilised, and stained with fluorescent antibodies specific for phosphorylated Syk and Akt, CD20, CD21, CD27 and IgM. Samples were analysed using flow cytometry.
- *Results*: The rate of Syk phosphorylation was increased with the addition of C3d, compared to IgM stimulation alone. Akt phosphorylation peaked with Syk phosphorylation after IgM and C3d stimulation, and was greatest after 30 minutes. Stimulation with C3d alone did not induce Akt or Syk phosphorylation.
- *Discussion*: When adult B-cells receive stimulation through their BCR and CD21 the rate of phosphorylation is enhanced compared to BCR stimulation alone, while C3d stimulation did not induce signalling. In future experiments, the early childhood B-cell response to BCR and CD21 stimulation will be characterised. It is hypothesised that signalling through CD21 is impaired during the first two years of life, thus explaining the irresponsiveness to polysaccharide antigens.

INTRODUCTION

- B-cells from an adult immune system can respond to non-protein antigens, including blood-group (ABO) polysaccharides, in the absence of T-cell help
- Children have an impaired ability to respond to blood-group polysaccharides within the first two years of life
- During this time, blood-group incompatible heart transplants can be successfully performed, leading to persistent, donor-specific immune tolerance . The mechanism of tolerance has yet to be elucidated
- Complement receptor 2, CD21, comprises part of the B-cell co-receptor, binds to complement fragment C3d, and is crucial in the response to polysaccharide antigens
- In this experiment, our aim was to determine how C3d induced stimulation of CD21 impacts the kinetics of the B-cell receptor (BCR) intracellular signaling cascade

MATERIALS AND METHODS

Cell Preparation

- Frozen, adult volunteer peripheral blood mononuclear cells were thawed and rested in culture media overnight in a 37°C incubator prior to the experiment

Phosphorylation Flow Cytometry

- Intracellular protein phosphorylation events were analysed using BD Phosflow™ cell signalling flow cytometry

Phosphorylation Flow Cytometry

- Cells were stimulated with 8µg/mL biotinylated anti-IgM antibodies, with or without the addition of 3µg/mL of purified C3d
- Cells were fixed 30 seconds to one hour after stimulation, permeabilised, and stained with fluorescent antibodies
- Fluorescent antibodies were specific for: CD20, CD21, CD27, IgM, phosphorylated Syk and phosphorylated Akt; cells were analysed using a FACS-Canto® II flow cytometer

RESULTS

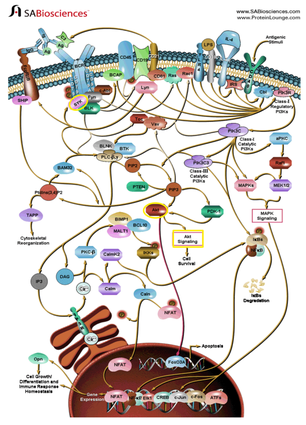

Figure 1: A flow diagram of B-cell intracellular signaling in response to B-cell receptor and co-receptor stimulation

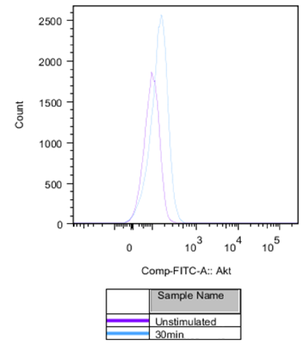

Figure 2: Comparison of the fluorescent intensity of FITC conjugated antibody specific for the phosphorylated form of Akt in an unstimulated sample and a sample 30 minutes post-IgM and C3d stimulation

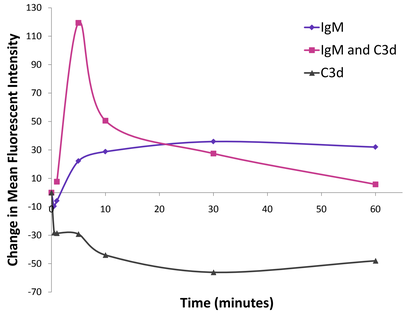

Figure 3: Comparison of the change in Mean Fluorescent Intensity (MFI) of the PE conjugated antibody specific for the phosphorylated form of Syk as a function of time after stimulation with either IgM (blue diamonds), IgM and C3d (pink squares) or C3d alone (grey triangles)

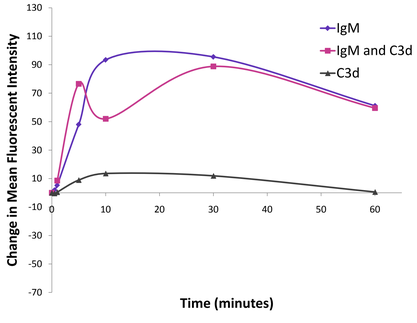

Figure 4: Comparison of the change in MFI of the FITC conjugated antibody specific for the phosphorylated form of Akt as a function of time after stimulation with either IgM (blue diamonds), IgM and C3d (pink squares) or C3d alone (grey triangles)

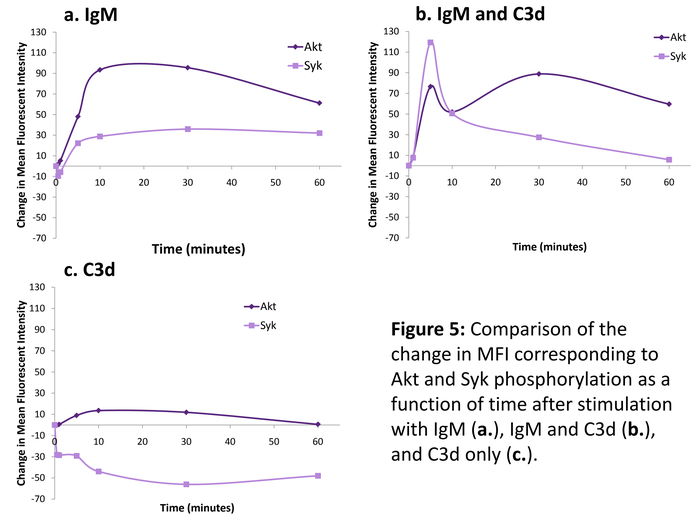

Figure 5: Comparison of the change in MFI corresponding to Akt and Syk phosphorylation as a function of time after stimulation with IgM (**a.**), IgM and C3d (**b.**), and C3d only (**c.**).

- Syk phosphorylation is greater and faster in IgM and C3d stimulated cells compared to IgM stimulation alone (Figure 3, Figure 5a)
- Akt and Syk phosphorylation peaks at 5 min during IgM and C3d stimulation, and reaches a secondary maximum peak after 30 minutes (Figure 2, Figure 4, Figure 5b)
- C3d stimulation alone does not induce Akt phosphorylation and decreases Syk phosphorylation (Figure 5c)

SUMMARY

- Co-stimulation of CD21 with complement split product C3d enhanced BCR intracellular signaling by increasing the rate of Syk phosphorylation; whereas CD21 activation without stimulation of the BCR reduces the level of phosphorylated Syk. This may prevent activation of B-cells that are not bound to their specific antigen.
- C3d co-stimulation of CD21 results in early enhancement of the BCR signal via Syk and Akt phosphorylation, whereas IgM stimulation alone causes slower but longer persisting phosphorylation of Syk.
- In future experiments we will investigate the early childhood response of peripheral blood mononuclear cells to IgM and polysaccharide stimulation, with and without C3d addition.
- It is hypothesized that the intracellular signaling cascade is impaired in B-cells for the first two years of life, corresponding to the period of irresponsiveness to polysaccharide antigens.
- Differences in B-cell co-receptor function could explain why children tolerate a blood-type incompatible heart transplant within the first two years of life.

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