Assessment of JAK/STAT inhibition as a therapeutic target in Primary Biliary Cholangitis (PBC)

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Introduction:

Primary biliary cholangitis (PBC) is a chronic, T-cell mediated autoimmune disease. It is characterised by the destruction of bile ducts leading to an accumulation of bile, inflammation and scarring of the liver as shown in Figure 1 [1]. As a consequence of excessive scarring, patients face liver failure and require liver transplantation.

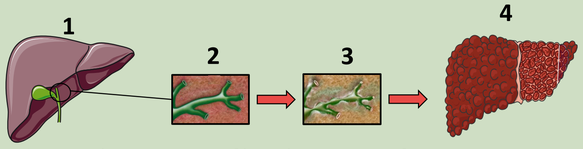


Figure 1: The progression of liver deterioration of a PBC patient.

Ursodeoxycholic acid (UDCA) is the only drug licensed to treat PBC in the UK. However, it does not improve the mortality rate and is also ineffective in a third of PBC patients, particularly the male and pre-menopausal female population [2][3]. Stressed biliary epithelial cells (BEC) have been shown to produce signalling molecules called cytokines (e.g interleukin (IL)-23) that favour the maturation of T helper (Th) 17 cells [4]. Th17 cells have been associated with poorer prognosis in PBC and other autoimmune diseases [5]. We hypothesised that the inhibition of the cytokine receptors using JAK/STAT inhibitors might be able to control this autoimmune reaction as shown in Figure 2.

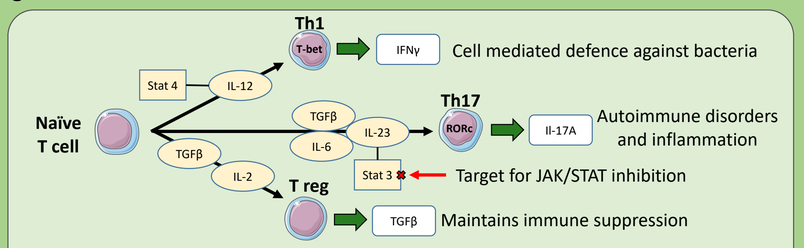


Figure 2: Differentiation of CD4+ T-helper subsets is determined by their respective cytokines.

Aims:

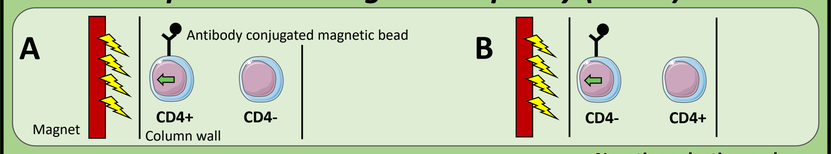
- Optimise and establish a cell model of PBC that could allow JAK/STAT inhibitors to be tested in the laboratories.

Methods:

- Peripheral blood mono-nuclear cells (PBMC) were collected from the blood of healthy volunteers.
- CD4+ cells (Th cells) were separated from the PBMC (positive vs negative selection) and polarised to produce either Th1 or Th17 cells using their respective cytokines (addition of cytokines vs CellXVivo kit).
- BEC were subjected to stress using 200µM hydrogen peroxide (H_2O_2) and put into co-culture with the CD4 and Th 1/17 cells.

Results:

Negative selection with double column filtration of CD4+ cells produced the greatest purity (>95%).



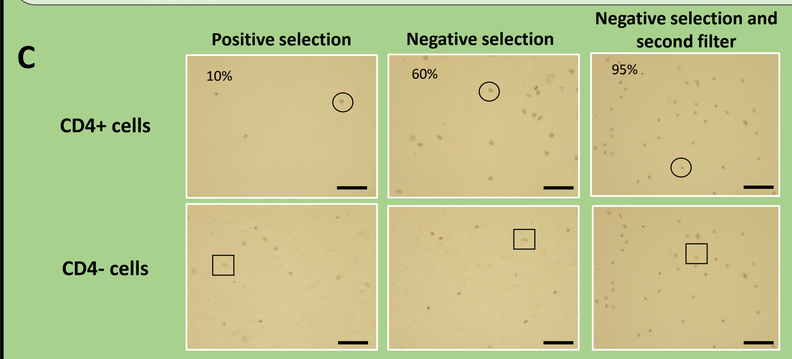


Figure 3: (A) Positive Selection. (B) Negative selection (C) CD4 cell smears as shown by immunohistochemistry. Cell counts were performed at x20 magnification. Positive staining (CD4+ cells) were shown as brown as indicated by the circles. CD4- cells are indicated by the square boxes. Scale bars represent 100µm.

While the CellXVivo kit successfully induced the T-bet transcription factor, IL-17A production was also increased, indicating that Th17 cells were also present in the population.

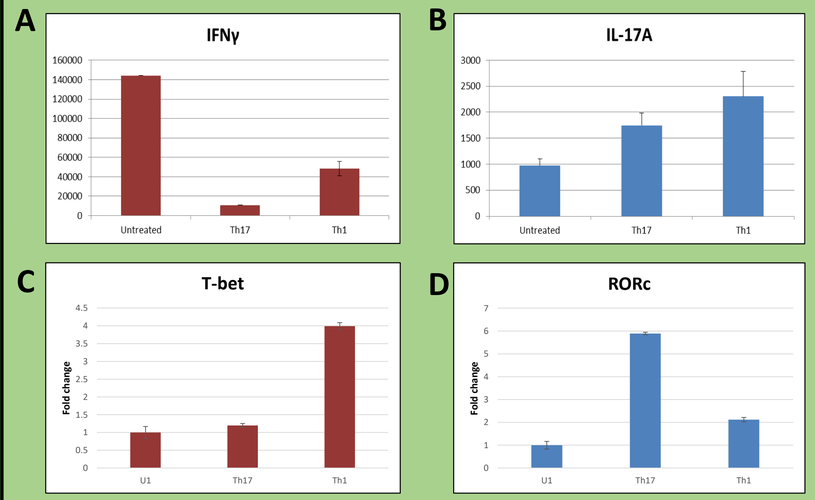


Figure 4: (A-B) Graphs represent production of Interferon-gamma (IFNγ) and IL-17A respectively by the Th cell subsets. **(C-D)** Graphs represent expression of transcription factors T-bet and RORc in the Th subsets.

Th1 cells take up less IL-6 from the stressed biliary epithelial cell environment compared to the CD4+ cells.

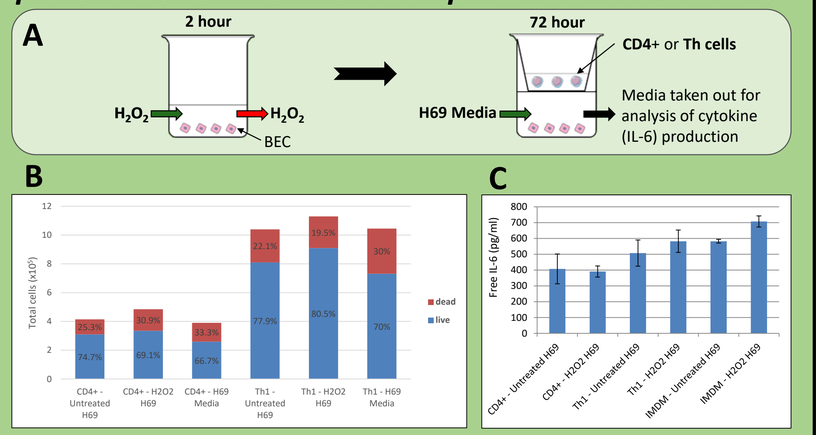


Figure 5: (A) Diagram of the T cell/BEC co-culture model. **(B)** Percentage viability and total number of T cells after 72 hours in co-culture. **(C)** Levels of free IL-6 in the H69 media after 72 hours.

Conclusion:

- Negative selection of CD4+ cells using the Miltenyi kit and filtration through two columns was the most reliable method of obtaining a high yield of pure CD4+ cells.
- Adding cytokines to the CD4+ cells directly was more reliable than the CellXVivo kit at Th1 cell differentiation.
- Differentiated Th 1 cells are less capable of taking up cytokine compared to the naïve CD4+ population. This suggests that JAK/STAT inhibitors would be effective as an earlier treatment to target differentiation of naïve cells.

Future work:

- Assessing the effects of commercially available JAK/STAT inhibitors (AG490, AZD1480, CP-690550, CYT387 and Ruxolitnib) on the model.

References:

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Acknowledgements:

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