BCL-3 a 'master regulator' of CD4+ T cell dysregulation

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1 Introduction

- Rheumatoid arthritis (RA) is disease in which a particular type of immune cell called a CD4+ T "helper" cell is thought to "misbehave" to cause autoimmunity – where "friendly fire" by the immune system damages the body.
- As a result RA patients suffer painful inflammation of the joints and irreversible joint damage may occur.
- It has been found that a gene called BCL-3 is "switched on" in circulating T helper-cells of early RA patients.
- We hypothesise that the resultant high levels of BCL-3 protein could be responsible for causing these cells to misbehave, acting as a 'master regulator' of T helper cell dysregulation.
- The aims of this project were to establish whether the over-production of BCL-3:
  - Has protective properties over the death (known as apoptosis) of T helper-cells.
  - Results in increased production of interleukin 2 (IL-2) which is a signalling molecule produced by T-cells when activated by a pathogenic body, such as a microbial infection.
- These experiments could potentially identify BCL-3 as an important target for future RA treatments that might switch off the irreversible consequences of T helper cell misbehaviour.

2 Methods

Cell lines

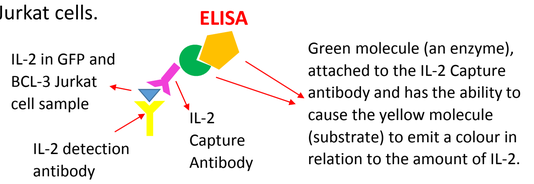
- During this research project laboratory experiments were carried out using a Jurkat human T-helper cell line which had been manipulated to over-produce the mouse BCL-3 protein along with green fluorescent protein (GFP). A Jurkat cell line only over-producing GFP, not BCL-3, was used as a comparator control.
- Characterisation of the Jurkat cell lines was carried out using fluorescent microscopy, quantitative real-time PCR, and western blot to look at the expression of BCL-3 at the molecular level as well as at the protein level.

Cell death

- Flow cytometry was used to determine the level of cell death (apoptosis) following treatment of the Jurkat cells with a signal (anti-Fas antibody) that causes the cells to die.

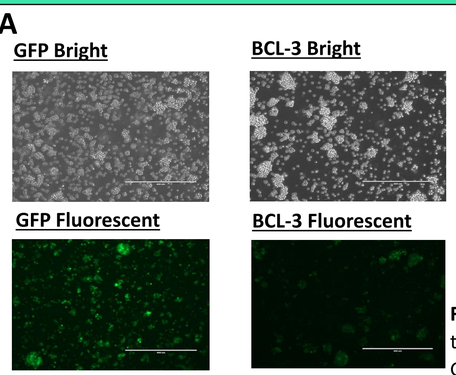
IL-2 production

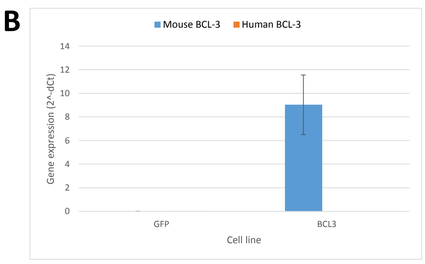
- IL-2 was measured using an ELISA following activation of the Jurkat cells.



Results and Discussion 1

3 Characterisation of the cell lines





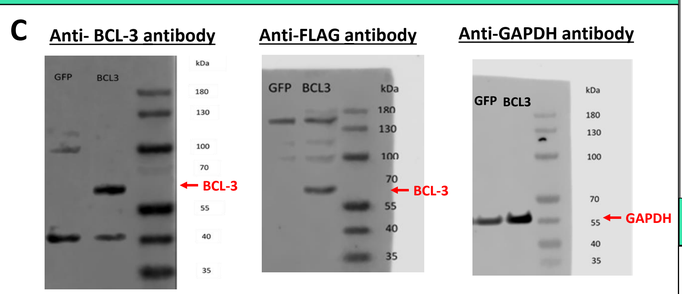


Figure 1: Characterisation of the Jurkat cells lines. Jurkat cells over-producing the BCL-3 protein along with GFP (BCL-3) and Jurkat cells only over-producing GFP (Green Fluorescent Protein) were assessed for GFP production using bright and fluorescent microscopy (**A**). (**B**) Quantitative real-time PCR was carried out to confirm that mouse BCL-3, and not human BCL-3, was over-produced at the molecular level in the BCL-3 Jurkat cells and was not present in the GFP Jurkat cells. A duplicate of each sample type was measured. (**C**) A western blot was performed on cell lysates to confirm that BCL-3 protein was being produced in the BCL-3 Jurkat cells and not in the GFP Jurkat cells. BCL-3 was only present in BCL-3 Jurkat cells (band at ~55kDa) (left blot). The BCL-3 protein was tagged with a FLAG sequence and the anti-FLAG antibody only recognises a band in the BCL-3 Jurkat cells (band at ~55 KDa) (middle blot). An anti-GAPDH antibody showed that the samples were loaded at equal amounts.

Discussion: The BCL-3 Jurkat cell line over-produces BCL-3 at both the molecular and protein level.

Results and Discussion 2

4 Cell Death

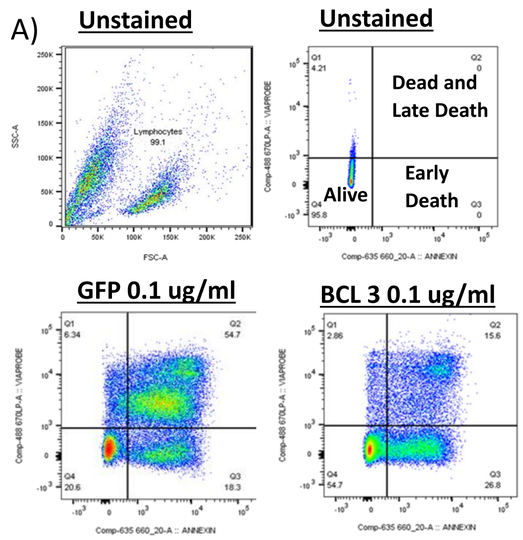


Figure 2: Over-production of BCL-3 in T cells may protect cells from cell death. To test the hypothesis that over-production of the mouse BCL-3 has protective properties and prevents a cell from undergoing cell death, cell viability experiments were performed and measured using a flow cytometry assay on a BDFACSCantoII (a machine used to measure cell death). Different concentrations of a signal that kills cells, known as anti-Fas antibody, was given to the cells to cause cell death, which was detected the next day by staining each Jurkat cell type with dyes identifying dead cells. Cells were categorised as Alive, Early Death, Late Death and Dead as shown in (**A**). (**B**) A summary of the results obtained from 5 independent experiments.

Discussion:

- Jurkats over-producing BCL-3 have a greater proportion of cells that are alive and in early death, and less dead cells, compared to the control GFP Jurkat cells.
- This implies that the over-production of mouse BCL-3 in Jurkat cells has protective properties against cell death.

5 IL-2 production

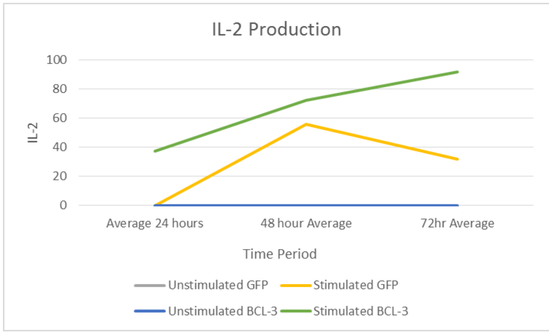


Figure 3: Over-production of BCL-3 in T cells may increase the production of IL-2. Jurkat cells were given a signal (stimulated) to produce IL-2. The amount of IL-2 produced was measured using an ELISA after stimulation for 24, 48 and 72 hours. The results are from **2** independent experiments.

Discussion:

- The stimulated BCL-3 Jurkat cells produce a greater concentration of IL-2 after 24, 48 and 72 hours, with the greatest difference being after 72 hours.
- Over-production of BCL-3 causes a greater production of IL-2

6 Conclusions

The project has revealed that:

- The over-production of the BCL-3 gene has protective properties over the process of cell death, and this resistance to cell death could contribute to T helper cell misbehaviour.
- Furthermore over-production of the BCL-3 gene causes an increase in the production of IL-2 by T cells, which could also contribute to misbehaviour of the cells.
- Future work - further repeats of the experiments need to be made
  - further viability experiments need to be carried out for different time periods