University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

   Surname: Martin Forename: Claire

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2. Supervisor:

   Surname: Milling Forename: Simon W F

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3. Research Project Report

   3.1 **Project Title (maximum 20 words):**

   Can dendritic cells drive Th2 responses when exposed to antigens derived from the parasite *Schistosoma mansoni*?

   3.2 **Project Lay Summary (copied from application):**

   Over 240 million people suffer from schistosomiasis, a socioeconomically devastating parasitic disease. After infection, eggs are produced and become trapped in the small intestine and liver, driving a chronic immune response that can cause debilitating,

eventually fatal loss of organ function. Although this response has been well characterized, how it is initiated has not.

We have developed a mouse model that replicates important elements of the early immune responses against Schistosome eggs in the intestine. We are studying how antigen presenting cells (APCs), which alert the immune system, detect Schistosome eggs and activate other immune cells, inducing the ensuing responses.

3.3 **Start Date:** 9/06/2014 **Finish Date:** 1/08/2014

3.4 **Original project aims and objectives (100 words max):**

1. Establish a positive control for Th2 differentiation of naïve T cells using *in vitro* culturing with optimum cytokine stimulation. This would show that the Th2 subset can be induced under ideal conditions.
2. *In vitro* culturing and antigen stimulation of bone marrow dendritic cells (BMDCs). Effects of stimulation would be investigated through changes in costimulatory marker levels.
3. *In vitro* co-culture of antigen stimulated BMDCs and naïve CD4 T cells. Investigation of T cell responses was expected to show that LPS stimulated BMDCs polarise T cells to the Th1 phenotype, and SEA stimulated BMDCs trigger the Th2 phenotype.

3.5 **Methodology: Summarise and include reference to training received in research methods etc. (250 words max):**

Initial training was undertaken in basic lab skills, ELISAs and analysis on Excel, flow cytometry data acquisition on the MACSQuant and analysis on FlowJo, and mouse organ harvests.

1. *In vitro* Th2 cells were cultured using FACS sorted naïve CD4 T cells from mice lymph nodes in various cytokine and culture length conditions. The cells were analysed by flow cytometry to check for proliferation and activation and the culture media supernatant analysed by ELISA for Th2 cytokines.
2. BMDCs were cultured using bone marrow cells in Flt3/R5 media before applying LPS, SEA or LPS and SEA antigen stimulation for 2 hours or overnight. BMDCs were then stained to analyse MHCII, CD40, CD80, CD86 and OX40L costimulatory marker expression levels by flow cytometry.
3. BMDCs were cultured and stimulated with antigen as before. They were then washed, OVA was applied for at least 4 hours and sorted OT-II naïve CD4 T cells were added. Ratios of 1:4 and 1:8 BMDCs:T cells were used in the 6 day co-culture and 1:2 and 1:8 in the 4 day co-culture. Th1 and Th2 subset positive controls were also used, providing optimum cytokine conditions for Th subset polarisation. CFSE stained T cells were used in some wells to check for proliferation. The appearances of the wells were checked by microscope over the course of culturing. FACS analysis was used to check for proliferation, activation and intracellular staining of IL4 and IFNγ. The culture supernatant was analysed by ELISA for IL4, IL5, IL13, IFNγ and TNFα.

3.6 **Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:**

1. After optimisation, the best results for Th2 differentiation were seen with coating wells with 4 μg/ml anti-CD3 and anti-CD28 and culturing in R5 media with 11 ng/ml IL2, 20 ng/ml IL4 and 10 μg/ml anti-IFNg for 4 days. An equal concentration of IL2 was added on day 2. Following washing and PMA/ionomycin treatment, ELISA analysis of the supernatant showed that the cells produced detectable IL4 levels which increased with cell number cultured in the samples (Figure 1A). Flow

cytometry showed around 8% of the $1x10^5$ cell number sample were activated (CD62L-) and had proliferated (Figures 1B and 1C). This proportion decreases with cell number.

2. In the final BMDC antigen stimulation experiment, cells were exposed to 200 ng/ml LPS, 25 µg/ml SEA or a combination of both for 18 hours and compared to unstimulated controls. Around 17% of cells were identified as MHCII+ CD11c+ dendritic cells. Looking at the costimulatory marker expression of these cells following antigen stimulation showed a distinct increase in CD40, CD80 and CD86 with LPS stimulation. SEA only stimulation primarily increased CD86 and, although slight, a greater increase in OX40L compared to other samples and controls (Figure 2A). Co-stimulation with SEA did not decrease LPS-related costimulatory marker upregulation.
3. ELISA results showed that the Th2 subset controls appeared to work well for IL5 production (Figure 3A), and fairly consistent moderate levels of TNFα could be seen in all wells, however the other ELISAs were less successful. The CFSE stained T cells of the LPS-stimulated-BMDC wells showed very high levels of activation and proliferation (Figure 3B). This was higher than activation and proliferation levels seen in the Th2 differentiation experiments. Unfortunately, the intracellular staining did not work.

3.7 **Discussion (500 words max):**

1. It was expected that these conditions would induce the Th2 subset as the anti-CD3 and anti-CD28 coating would provide activation and survival signals, and the cytokines would provide differentiation and proliferation signals. Although the activation levels were fairly low, Th2 cytokines could be detected, which must be in response to the culturing conditions applied. This proves our hypothesis correct, as

the coating and cytokines applied must have substituted for signals provided by interaction with an antigen presenting cell.

2. It is known that dendritic cells communicate with the cells they present antigens to through variations in costimulatory marker levels. Therefore it was expected that clear differences would be seen between the samples. This was the case, as distinct changes could be seen the costimulatory marker levels of LPS and SEA only stimulated dendritic cells, although stimulation with both LPS and SEA did not affect LPS-driven responses. This shows both that costimulatory marker levels are influenced by antigen stimulation and that other methods also exist for dendritic cells to communicate with the T cells they interact with that were not tested for, such as cytokine signalling.
3. It was predicted that the Th1 control and LPS-stimulated-BMDC samples would show high levels of IFNγ, and perhaps the pro-inflammatory TNFα, and Th2 control and SEA-stimulated-BMDC samples would show high levels of IL4, IL5 and IL13. Although IL5 was detected in the Th2 controls, the IL4 detected was likely from that added at the start of culturing and the IL13 levels detected were unreliable. The IFNγ ELISA plate was overdeveloped, therefore no comparisons could be made and no ELISA data was available for the Th1 control. TNFα was seen in all samples but it is uncertain whether this was produced by T cells or dendritic cells. Although the Th2 subset control worked, in order to acquire accurate Th1 results, the samples should be diluted before testing for IFNγ by ELISA again.

   There was only time to analyse the T cells of two wells for proliferation and activation, so the LPS-stimulated-BMDC wells were chosen as these were expected to show high results for both. The flow cytometry data for these T cells showed very high activation and proliferation in both co-culture samples. This shows that this co-culture model does work, as only the dendritic cells were in contact with the antigen

stimulation and have activated the T cells and induced proliferation. This was in the absence of any optimising cytokines or coating (aside from IL2 added to all samples to aid T cell proliferation) used in the Th subset polarising controls.

As in the ELISAs, high IFNγ and IL4 levels were expected for the Th1 controls and LPS samples and the Th2 controls and SEA samples respectively. However, the results from this were inconclusive and some wells had a very high level of cell death. This protocol still needs to be optimised and repeated with far more conditions and duplicates such that the effects of cell death can be reduced and accurate comparisons made.

4. **Reflection by the student on the experience and value of the studentship (300 words max):**

This project was the most challenging thing I have ever done but I really appreciated the experience. I don't feel that anything else could have given me a better idea of what working in science is actually like. As well gaining practical and analytical skills that will be useful for both my degree and beyond, I was taught the right type of attitude to have in research. The setbacks experienced and all the trial and error required felt frustrating at first but it was important to learn from them and move on, and I'm sure that this will really help my confidence. After being overwhelmed at first by all the new techniques it felt so rewarding to get to the stage where I could do many by myself.

The lab was a wonderfully open environment to work in, where people would always be willing to help you out or talk about what research they were doing. I was able to take part in the weekly lab presentations and journal clubs, which were valuable learning experiences. I would encourage anyone thinking of applying to do so. It's easy to feel incredibly daunted as an undergraduate trying to get experience like this but it really is worth it and people do want to help you. A summer project is the best way to decide if a career in research is what you want to do.

I am very grateful to Johannes Mayer and Simon Milling for letting me do this project and for helping me throughout it. I would also like to say thank you to the Head of College Scholars List Scheme for providing this opportunity.

5. **Dissemination: (note any presentations/publications submitted/planned from the work):**

I presented my data at a lab meeting, to an audience of tenured staff, post-doctoral researchers and PhD students.

6. Signatures: Supervisor  Date 26/8/14 Student  Date 26/08/14

Appendix 1: Th2 differentiation data

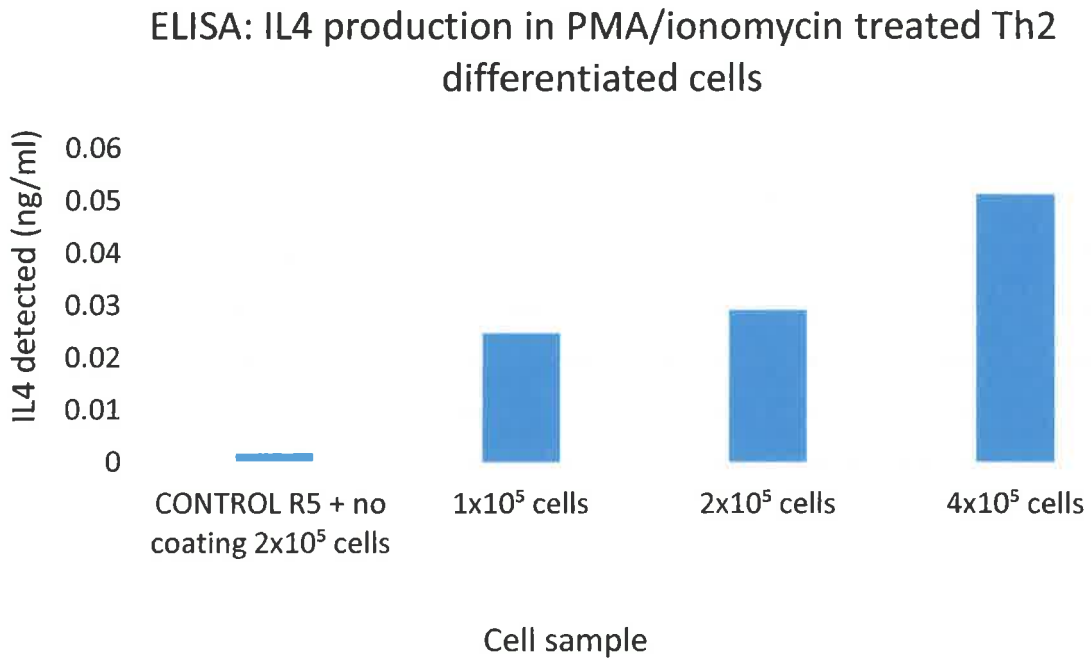

Figure 1A: *IL4 levels detected by ELISA in PMA/ionomycin treated in vitro Th2 differentiated cells. All samples were washed of culturing media prior to treatment to remove cytokines added at the start of culturing.*

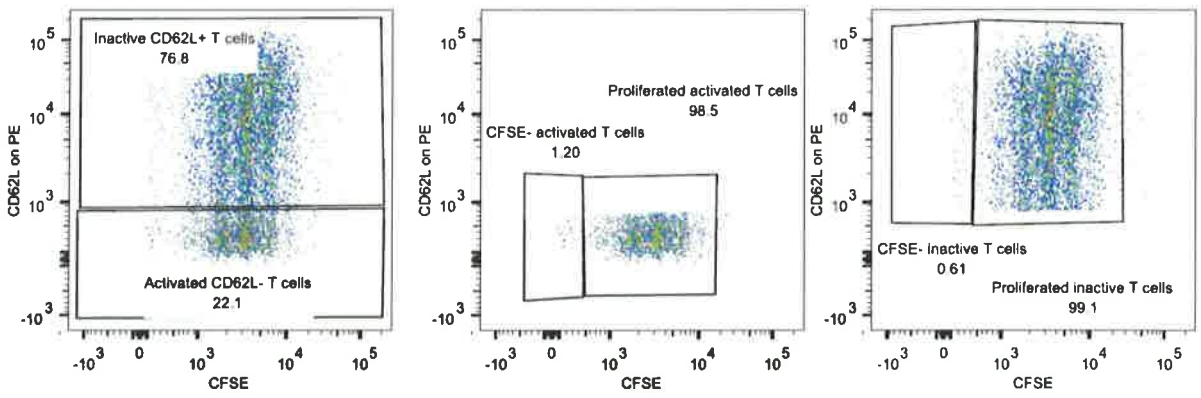

Figure 1B: *Flow cytometry data showing CFSE and CD62L expression of in vitro Th2 differentiated CD4 cells. Cells were gated to show live, single, CD4+ cells. CD62L- CD4 cells were gated as activated. The streaks of CFSE expression show cells that have taken up CFSE at the start of culturing and have proliferated, halving the amount of CFSE in each successive cell doubling.*

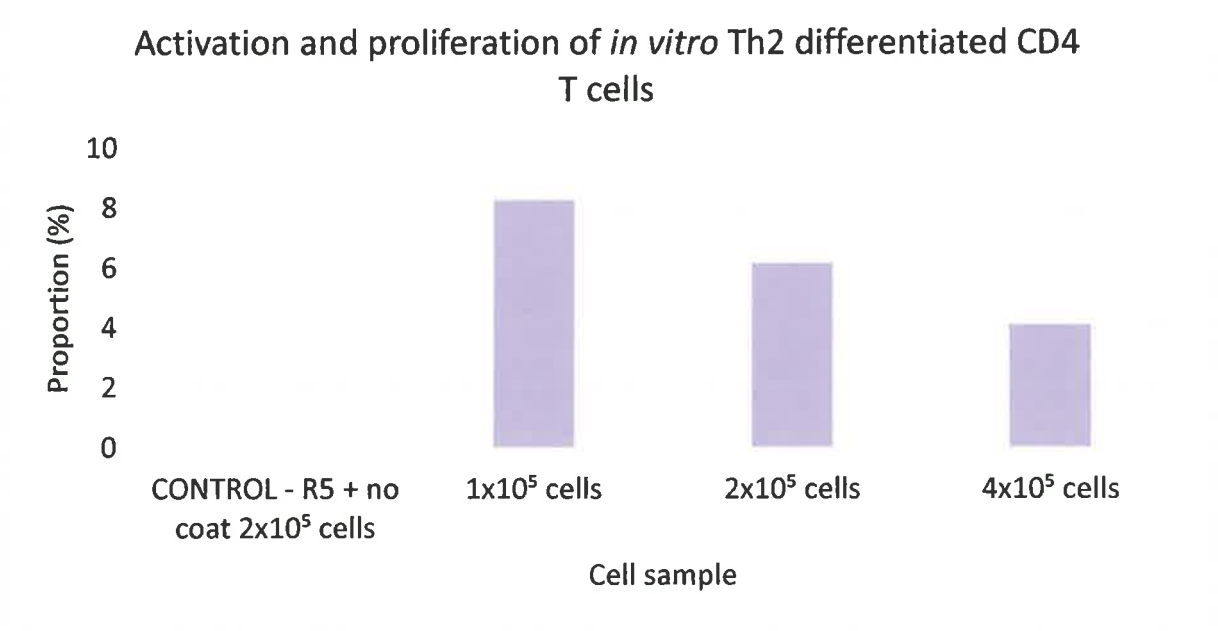

Figure 1C: *Proportion of in vitro Th2 differentiated CD4+ T cells that are activated (CD62L-) and have proliferated (based on CFSE expression).*

Appendix 2: BMDC culture and antigen stimulation data

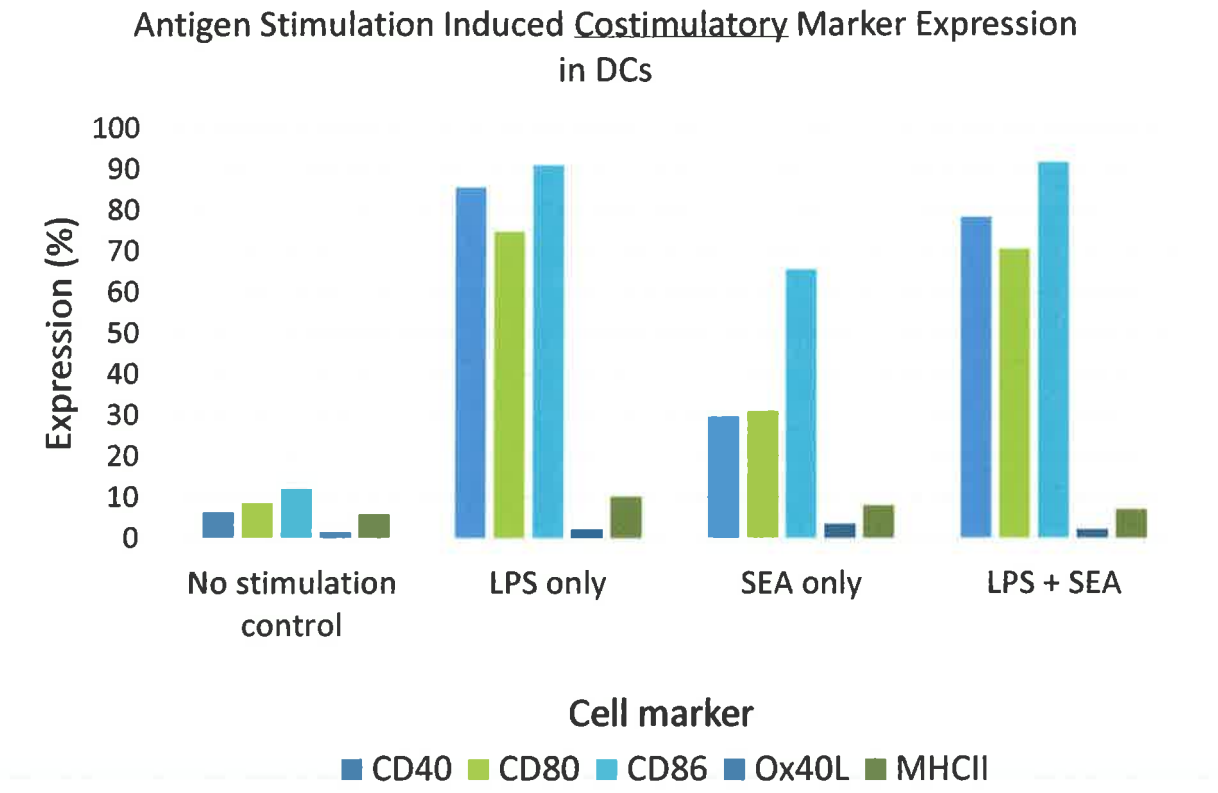

Figure 2A: *Flow cytometry data showing the relative expression of costimulatory markers in MHCII+ CD11c+ gated dendritic cells depending on antigen stimulation applied.*

Appendix 3: BMDC and naïve CD4 T cell co-culture data

Figure 3A: *IL5 levels detected in Th2 subset control wells of both co-culture plates. The 4 day sample was treated with Golgi Plug and the 6 day sample with PMA/ionomycin followed by Golgi Plug.*

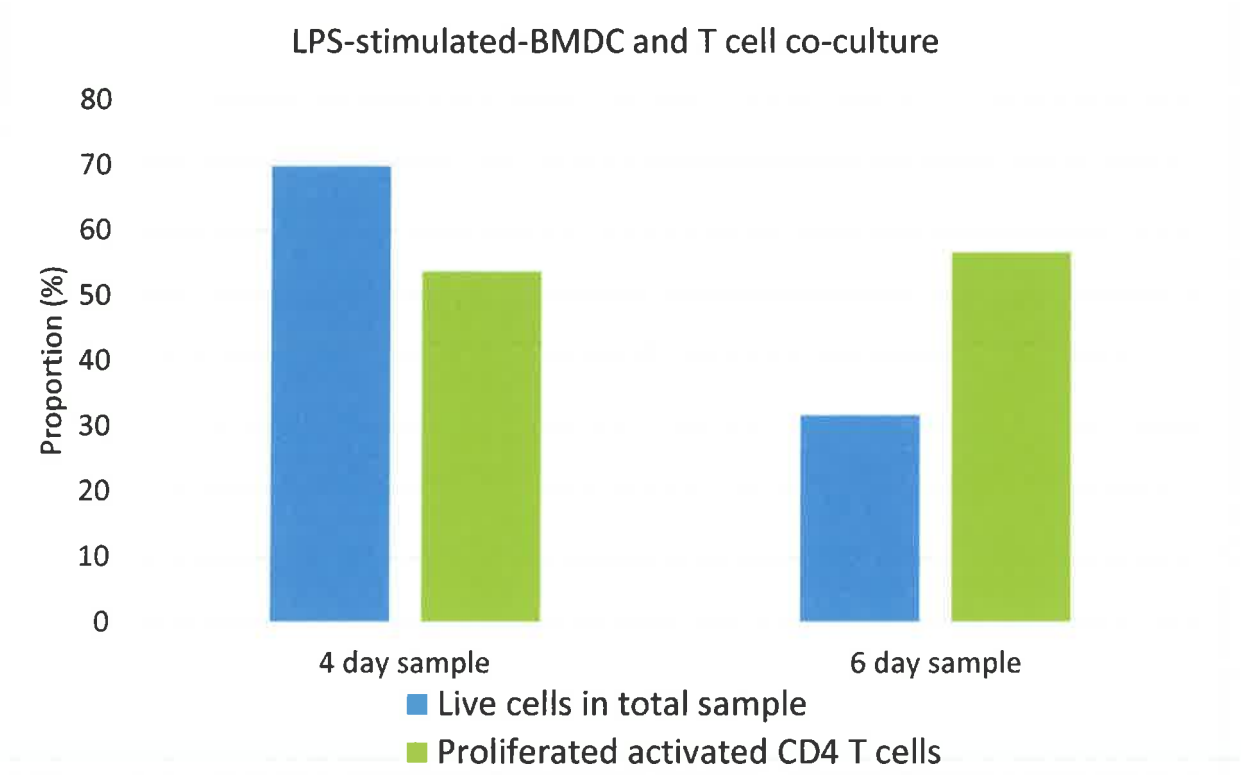

Figure 3B: *Flow cytometry data showing the proportions of live cells in total co-culture samples and the proportion of activated (CD62L-) and proliferated CD4+ T cells.*