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: Rogers Forename: Sophie

   E-mail address: 1101829R@student.gla.ac.uk

2. Supervisor:

   Surname: Goodyear Forename: Carl Steven

   E-mail address: carl.goodyear@glasgow.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   To investigate the effects of PI3 kinase inhibitors on the activation of CD8+ cells.

   3.2 Project Lay Summary (copied from application):

   Th1 cells (a subset of white blood cells) are known to be important in the process of inflammation and are thus implicated in many aspects of disease. Modulation of these cells could potentially lead to further understanding of the impact they have on health and disease and reveal novel ways to combat pathology. The proposed study will examine effects of signaling mechanisms on the development of Th1 cells.

However, it should be noted that upon starting my project the focus was shifted to CD8+ cytotoxic T cells rather than Th1 T cells.

3.3 Start Date: 08/06/2015 Finish Date: 31/07/15

3.4 Original project aims and objectives (100 words max): Modified to reflect a change in the type of cells to be investigated.

The aim of this project was to (a) investigate the effect of PI3 Kinase inhibitors on CD8+ T cells activation and (b) determine which isoform-specific inhibitor was most efficacious. Therefore, objective 1 was to establish a robust way of activating CD8+ T cells whilst objective 2 was to assess the effectiveness of PI3 kinase isoforms inhibitors on the activation of CD8+ T cells.

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

To perform experiments peripheral blood mononuclear cells (PBMCs) and CD8+ T cells had to be obtained from healthy donor blood samples. Training to isolate PBMCs from buffy coats through density gradient centrifugation was given and the procedure was carried out many times. Training using magnetic bead negative selection kits to isolate CD8+ T cells was also given. Once cells were isolated they were cultured (with appropriate training in tissue culture) and stimulated with various chemical and biological stimuli. No predetermined activation protocol was available and thus we tested numerous techniques

found in the literature including peptides, re-stimulation cocktails and chemical stimuli. I was taught how identify and follow protocols for chemical stimulation of cells: PMA and Ionomycin (BDBiosciences Cell Stimulation Cocktail), which acted as our positive control. I was also taught how to use biological stimuli (Miltenyi Biotech CMV PepTivators) and how to problem solve technical issues. The degree of cellular activation was measured using flow cytometry (MACsQuant analyser) and the data was analysed using Flowjo. I received training in both acquisition of data and subsequent analysis. In specific, the flow cytometry used two distinct markers of CD8 T cell activation: CD107a and interferon-gamma (IFN-g).

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

PBMCs were successfully isolated from human buffy coats. However, the activation of these cells (and more specifically the CD8 compartment) was challenging. Initially experiments used the commercially available CMV pp65 PepTivators (MACS Miltenyi biotec), which contain an immunodominant CMV peptide that is recognised by CMV specific CD8+ T cells. Importantly, CMV-specific CD8+ T cells are usually present at detectable levels in healthy adults. Unfortunately, the CMV pp65 PepTivators did not lead to a significant increase in IFN-g^+ $CD107a^+$ CD8+ T cells. It was therefore hypothesised that this was due to inadequate costimulation and to overcome this an antibody to CD28 was used in conjunction with CMV pp65 PepTivators. However, once again this did not lead to an increase in IFN-g^+ $CD107a^+$ CD8+ T cells. In an attempt to polyclonally activate CD8+ T cells we used a more generic activator: chemical cell

stimulation cocktail (eBiosciences). This resulted in a large activation signal, confirming cellular response and also cellular viability. Importantly, this polyclonal stimulation worked in both a PBMC and purified CD8+ T cell setting. These results confirmed that cells could be stimulated and upregulate IFN-g CD107a, but unfortunately the signal generated was too intense and non-specific to be of use in inhibitor studies. To determine if an alternative stimulation regime (that was permissive for inhibitor studies) was possible, further stimulants were tested. In brief, Concanavalin A (ConA), Phytohaemagglutinin (PHA) and CytoStim (MACS Miltenyi Biotec) were tested. The most significant level of activation, demonstrated by increased IFN-g levels, came after CytoStim exposure (see figure 1 in appendix). However, the level of CD107a (which should correlate with IFNg) was un-interpretable and thus true positive cells could not be identified (see figure 2 in appendix).

3.7 Discussion (500 words max):

Over the course of the placement a considerable amount of time was spent problem solving. Extra activation products, markers and additional co-stimulation antibodies were added and a modest improvement in CD107a and IFN-g was seen in some cases but this was not sustained or consistent throughout repeat experiments. It was suggested that the source of our cells may have been causing some of the issues we were seeing. Buffy coats are received come from the Scottish National Blood Transfusion Service (SNBTS). These are tested by the SNBTS for various diseases and this can delay release of the samples. It is therefore possible that these cells are up to two days old. This could have a dramatic impact of the ability of the CD8+ cells within the blood to

activate as they have been removed from the circulation for a reasonably long time. A way to test this theory would be to use fresher blood.

To identify activated cells several markers were used including CD107a, CD8 and later CD5. CD107a has been hailed as a very sensitive marker of degranulation. It is expressed on the cell surface during activation before being recycled. There are a number of papers that show increased levels of the protein following CD8+ activation in a similar manner to the method we used. However we were unable to detect the increased expression to the same degree. Distinguishing CD107a positive and CD107a negative CD8+ cells was difficult and requires further work. CD107a is expressed maximally prior to the CD8+ cells degranulating and releasing their contents and after this it internalises so is harder to detect. We did consider this in our protocol and introduced the antibody for CD107a into the samples prior to stimulation and kept it in for the duration of the experiment but still encountered issues. The last experiment conducted before the end of the studentship aimed to detect CD5 and granzyme. The rational for this is that CD8+ is known to be down-regulated once the cells have been activated which could be the reason why it has been difficult to detect cells that are CD8 and CD107a positive. CD5 is a more stable marker present on CD8+ cells, however, it is also present on other types of immune cells so staining for both would still need to be done and CD5 would act as a secondary marker to confirm the cells are CD8+. Granzyme is a potentially useful marker of activation as it is released upon CD8+ activation. The results from the first experiment with these two markers showed us that while granzyme is present in the stimulated culture, identifying the cell type producing it requires further research. From this protocol we were unable to confidently identify cells which were CD8+ and granzyme producers. Similar issues were encountered when examining the CD5+

stained cells. Though CD5+ cells were clearly present, defining CD8+ CD5+ cells was impossible as the boundary between CD5- and CD5+ CD8+ cells was unclear.

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

I feel that this placement has been beneficial for me for numerous reasons. Being able to be involved in a team with a link to both academia and industry has introduced me to an area of research unlike most others. I was invited to go to meetings with staff of various roles within a major pharmaceutical company and had one-on-one discussions with established researchers about my future. I now have a better understanding of the types of job and potential areas of further study my degree will enable me to enter once I graduate. I feel that this placement has improved my practical lab skills significantly and given me greater confidence when working independently. Prior to this placement my lab experience was quite limited but now I would feel confident using a range of equipment. I am particularly grateful for being taught how to use flow cytometry as it is rare for an undergraduate to get to use such a complex piece of technology. Another aspect of my placement which was beneficial was the end of month catch-up meeting the lab group and Director held. I was asked to present my findings up to that point on both occasions. Doing an informal but informative talk in this way started conversations about the directions the work could go in and let other people give their opinions on the

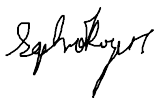
methods we were using. I also enjoyed getting to practise doing public speaking, which is an activity I have never really felt comfortable doing.

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

   The ultimate aim of the work carried out over the course of my placement is to test the mechanisms and efficacy of PI3 kinase inhibitors for therapeutic purposes. The group presently are working from a number of different angles with this aim, and will continue to do for as long as the results are positive and the company which commissioned the research feel it is viable.

6. Signatures:

   Supervisor  Date 17/08/15

   Student  Date 17/08/15

Appendix: Results

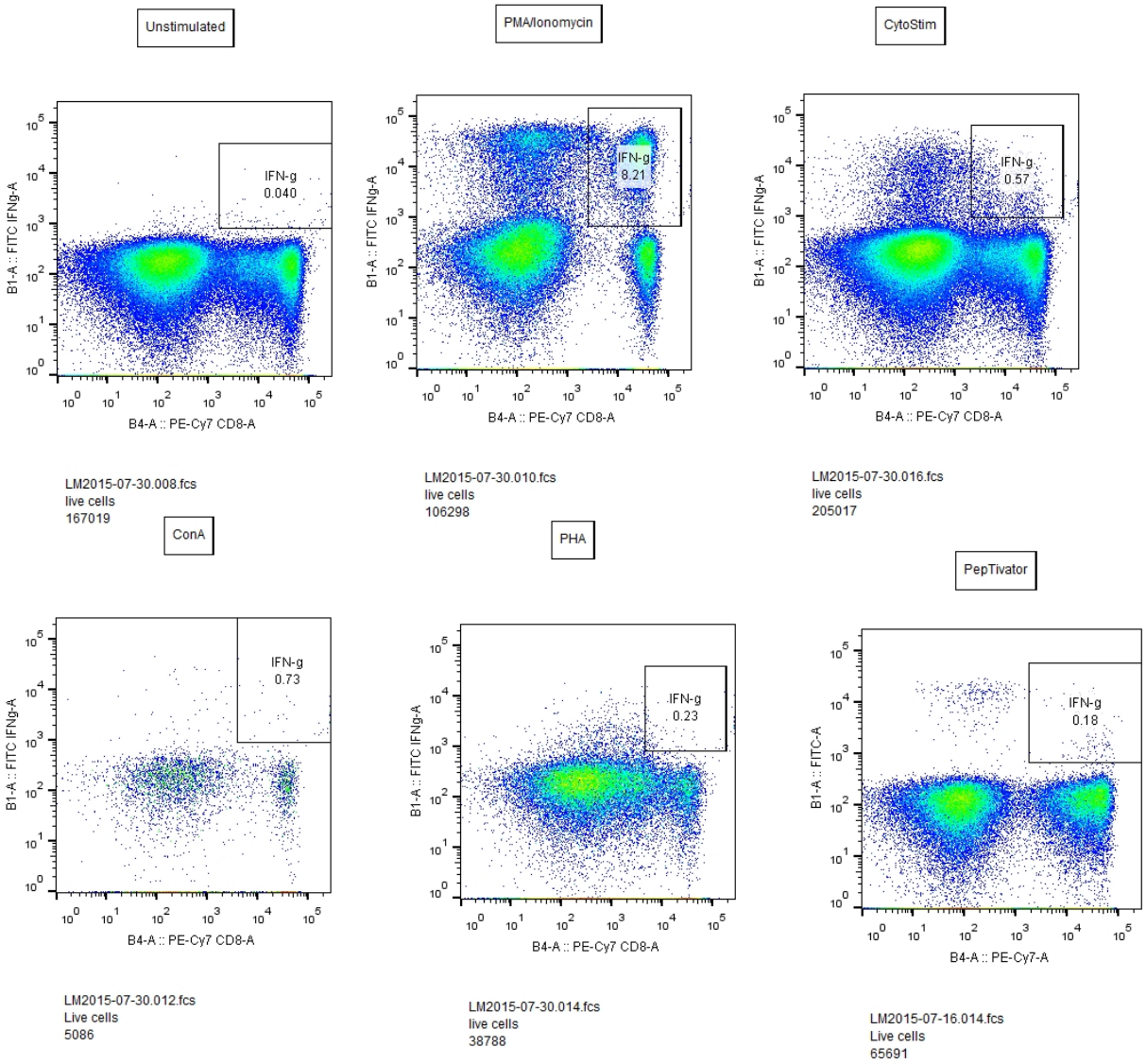


Figure 1: Examples of IFN-g staining. The cells positive for CD8+ which are producing IFN-g can be seen in the top right hand quadrant. The signal for PMA/Ionomycin is the largest as

would be expected. Activation of cells with CytoStim also produces a clear CD8+IFN-g+ signal. ConA, PHA and PepTivators lead to low levels of CD8+ and IFN-g+ positive cells

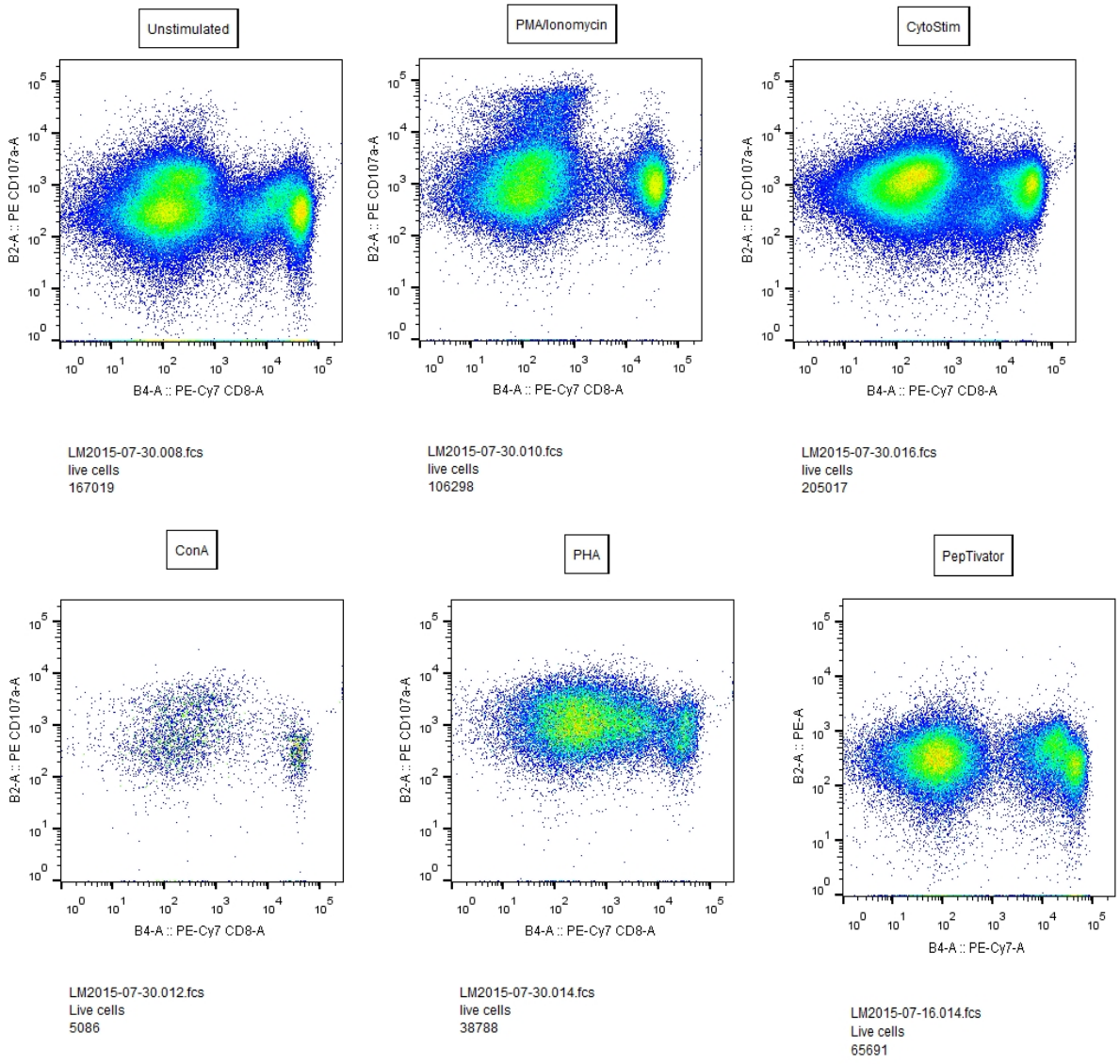


Figure 2: Examples of CD107a staining. From these images the difficulty in detecting the difference populations of cells can be seen. There are no clear quadrants like the ones visible in figure 1. Distinguishing between the cells positive for CD107a and those negative for CD107a will require more work.