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: Bart Forename: Valentina

E-mail address: 2087837B@student.gla.ac.uk

2. Supervisor:

Surname: Harnett Forename: Margaret

E-mail address: Margaret.Harnett@glasgow.ac.uk

3. Research Project Report

3.1 Project Title (maximum 20 words):

Investigation of the signalling mechanism(s) underpinning conversion of synovial fibroblasts to an aggressive phenotype during rheumatoid arthritis pathogenesis

3.2 Project Lay Summary (copied from application):

Rheumatoid arthritis is a chronic autoimmune disease in which our immune system starts attacking our joints causing inflammation and bone damage resulting in pain, disability and even early death. There is no cure and current treatments do not work for

all patients. During development of disease, cells called synovial fibroblasts, change such that by becoming hyper-responsive to proinflammatory mediators they cause joint damage. We are therefore trying to work out how this happens as by identifying the molecular mechanisms involved, we can design drugs tat stop or even reverse this process and hence cure arthritis directly in the joints.

3.3 Start Date: 10.08.2015 Finish Date: 18.09.2015

3.4 Original project aims and objectives (100 words max): The original core aim was to investigate the acute counter-regulatory signals by which IL-17 rewires healthy SF to an aggressive phenotype and whether this pathogenic switch is desensitised/reversed by IFNβ, focusing on STAT3- to STAT1-pathways. However, as data from this and other laboratories suggested that chronic exposure to IL-1β, a key regulator of IL-17 responses, epigenetically remodel SF to an aggressive phenotype that may promote such differential responses, it was decided to first characterise the mechanisms underpinning this to inform on how they may impact on the pathogenic switch driven by its downstream mediator, IL-17.

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

Synovial fibroblasts (SF) were derived by in vitro culture for 3weeks from the paws of naïve DBA/1 mice and those with collagen-induced arthritis (CIA), treated or not with S5 (CIA S5) a small molecule analogue of the parasitic worm product, ES-62 that both afford protection in this model. Cells were expanded in media containing 10% FCS and 1% Penicillin/Streptomycin and before stimulation were synchronised in DMEM 1% FCS overnight. For acute stimulation, cells were stimulated for 24 hours with IL-1β at 10

ng/ml and S5 at 100 ng/ml. SF from mice exposed to S5 in vivo were left untreated. Conditioned media was collected for analysis of cytokine production (IL-6 and CCL2) analysis by ELISA kits (eBioscience) and mRNA extracted (using Qiagen RNeasy kit) from the cells for analysis of matrix metalloproteinases, MMP9 and MMP13 by Taqman qRT-PCR (Life Technologies).

For chronic stimulation, cells in triplicate cultures were stimulated every day for 8 days using IL-1 1 ng/ml and/or S5 at 10 ng/ml, with the conditioned media collected throughout the time course. RNA was extracted from the cells at the end of the time course.

Data were analysed using Prism Graph Pad software.

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

1. Effect of chronic exposure to IL-1β on synovial fibroblast cytokine production

SF from naïve mice were cultured in vitro for 8 days to IL-1β and also with S5 to determine whether this protective agent could prevent their pathogenic remodelling. IL-1β known to exert its effects by inducing DNA methylation and here we wanted to assess whether this is associated with cell aggressiveness in terms of pro-inflammatory production of IL-6 and CCL2. S5 has been shown to mediate protective action in CIA by inhibiting IL-1β responses and thus we investigated whether S5 would also be able to inhibit IL-1β actions in terms of DNA demethylation and making naïve fibroblasts intrinsically aggressive. However, whilst it appears that compared to the unstimulated cells IL-6 production is indeed enhanced by the chronic exposure to IL-1β, S5 does not seem to be able to inhibit IL-1β effect. In the case of CCL2, production of which is

enhanced by IL-1β, it remains unclear whether S5 can inhibit the IL-1β response as the data obtained to date suggest that it may suppress this (Figure 1).

2. Effect of acute in vitro treatment with IL-1b on SF from naive and CIA mice, including those exposed to S5 in vivo

CIA SF and those exposed to S5 in vivo were treated in triplicate cultures with IL-1β for 24 hours (CIA1b), with control cells (eg CIA) left untreated (Figure 2). The aim was to evaluate whether in vivo treatment with S5 could protect the fibroblasts from subsequent IL-1β-induced demethylation and thus prevent the aggressive phenotype producing pro-inflammatory cytokines. While again, cytokine production appears enhanced by acute exposure to IL-1β, no protective effects of S5 can be observed, as no significant differences could be found in either IL6 ($t(4)=1.544, p=0.1974$) or CCL2 ($t(2.574)=0.03956$, $p=0.9713$) concentration comparing CIA and S5 cells treated with IL-1β.

In addition, we wished to evaluate whether IL-1β induces SF aggressiveness in naïve fibroblasts by up-regulating MMP expression and if this could be prevented by in vivo treatment with S5. Indeed, MMP9 and MMP13 were both observed to be higher in cells treated with IL1β than in untreated controls. Concerning MMP9, the levels were significantly lower in SF derived from mice treated with S5 in vivo relative to those from control mice with CIA cells. Screening for MMP13, the same trend could be observed, with levels being highest in CIA cells exposed to IL-1β, followed by S5 cells treated with IL-1β, suggesting in vivo that treatment with S5 in fact does enable fibroblasts to, to a certain degree, resist to changes induced by IL-1β (Figure3 and 4).

3.7 Discussion (500 words max):

In the investigation of the levels of IL6, CCL2 produced by SF in response to acute or chronic treatment with IL-1b, the latter to mimic the effects of pathogenesis in vivo, it was hypothesised that pro-inflammatory cytokine release would be higher in SF exposed to IL-1β, as IL-1β has previously been shown to induce demethylation resulting in a rheumatoid arthritis phenotype, reflected by up-regulation of pro-inflammatory cytokines (Nakano, Boyle & Firestein, 2013). Thus, the high level of cytokines observed in all groups of naive SF at the start of the time course was suggested to be induced by the change in media from 1% FCS to 10% FCS leading to an activation of the cells and resulting in increase of all cytokine levels and metabolic functions. This would appear to be likely as the levels of IL6 produced by untreated naïve cells decreased rapidly, with no IL6 detected from day 6. The hypothesis that the cytokine production by naïve SF treated with a combination of IL-1β and S5 would be lower than that by SF treated with IL-1β alone was not confirmed, as essentially identical kinetics of production of IL-6 could be observed under both conditions. However, the conditioned media was only collected over 8 days of treatment, which might not have been long enough for modulation of responses to be clear. Further, S5 might have been too weak at the concentration used to counteract IL-1β, which should be considered in future studies. Also, it might be the case that S5 is only prophylactically and not therapeutically active against IL-1β -induced effects, and it could be interesting to start treatment with S5 before exposing the cells to IL-1β to test this theory. Alternatively, there is the possibility that S5 is only able to inhibit IL-1β production and might not have the capacity to counteract effects induced by IL-1β although the data for CCL2 suggested that S5 might be able to counteract at least some of the effects of IL-1β.

Indeed, comparison of the levels of MMP mRNA in SF from naïve, CIA (untreated or 24 hours with IL1β) and S5/CIA (untreated or 24 hours IL1b) mice showed that while no significant difference could be found comparing levels of MMP9 in naïve and S5 cells, SF

from mice exposed to S5 in vivo had lower levels of MMPs than the CIA disease controls ($p<0.01$). Moreover, MMP9 and MMP13 levels were observed to be significantly lower in IL-1β-treated SF from CIA mice exposed to S5 in vivo than in control CIA SF exposed to IL-1β. These results are consistent with the in vivo protection afforded by S5 in the CIA model, and indicate that in vivo treatment of SF with S5 is in fact able to provide a certain degree of resistance against IL-1β effects and that S5-based drugs might have potential in the treatment of RA. This is because MMPs, which normally are involved in tissue remodelling have been shown to play a major role in the pathology of RA through inducing degradation of the extracellular matrix in joints causing destruction and deformation (Visse & Nagase, 2003). Consistent with this, their release from fibroblasts is enhanced by IL-1β (Brennan & McInnes, 2008), a pro-inflammatory cytokine overexpressed in RA synovial fluid and plasma. A molecule being able to down-regulate MMP release even in the presence of high concentrations of IL-1β could potentially be able to prevent joint destruction and would therefore make an interesting candidate for development of RA medication, as treatment options at the moment are limited.

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

Having had no previous experience of working in a laboratory, this placement was challenging on many levels as, in addition to being introduced to a range of laboratory skills and techniques, , I learned the importance of planning ahead while learning how to deal with failure and disappointments. Indeed, the whole process not only gave me insight into working in a lab, but also, despite the short duration, has had a great impact on my personal development. Being able to plan experiments in my own time and work independently has given me confidence in my own laboratory and transferable skills, which I hope to expand on in future placements and that will certainly be helpful not only in my studies, but also in a

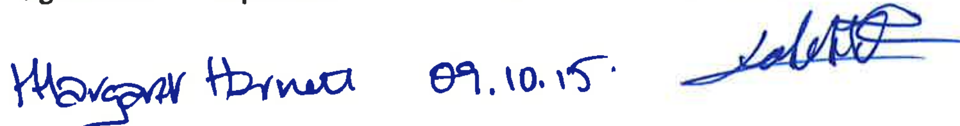
later career. It certainly was a pleasure to work in such a friendly and helpful environment and I hope to be able to go back one day.

I would like to thank Prof. Harnett for taking me in and enabling me to do this placement, Marlene Corbet for her patience in explaining procedures and answering all my questions, as well as the Head of College Scholars Scheme for sparking the idea in the first place and the financial support.

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

Marlene Corbet has planned further experiments to validate and extend these studies for future publication/dissemination.

6. Signatures: Supervisor Date Student Date



Figures:

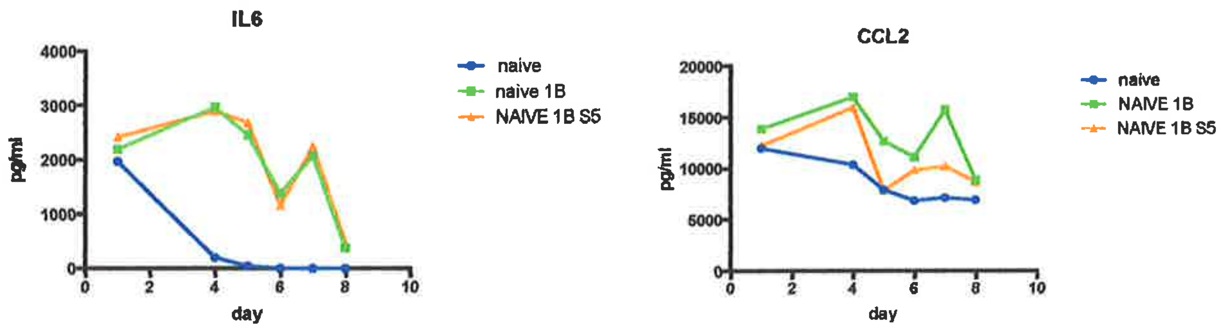


Figure 1: IL-6 and CCL2 production during chronic exposure to inflammatory molecules.

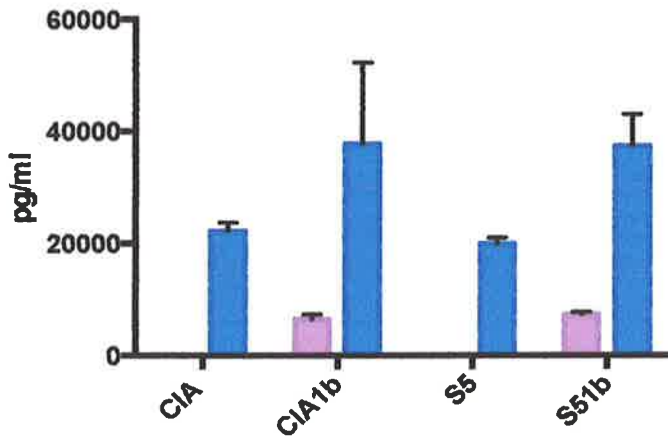


Figure 2: IL-6 (pink) and CCL2 (blue) production during acute exposure to inflammatory molecules.

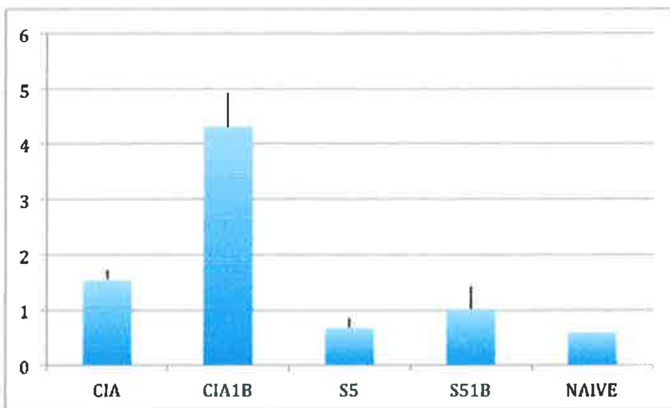


Figure 3: MMP9 mRNA production during acute exposure to inflammatory molecules.

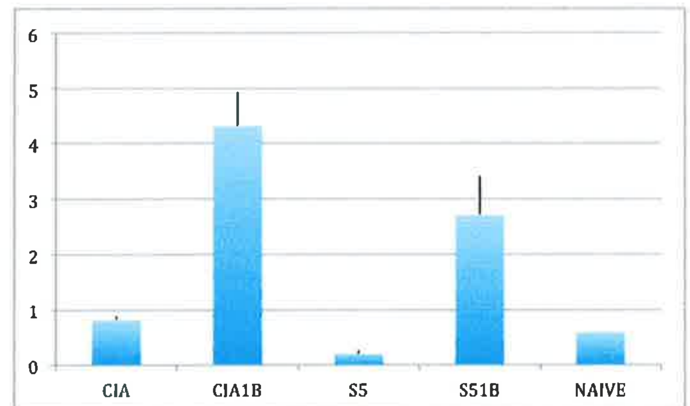


Figure 4: MMP13 mRNA production during acute exposure to inflammatory molecules.