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: Alonso Naranjo  Forename: Omar

   E-mail address: 2205810a@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 (16):

   Investigation of the impact of ES-62 on the microbiome in inflammatory disease and the ageing process.

   3.2 Project Lay Summary (copied from application):

   High fat diet (HFD)-induced obesity induces chronic inflammation that additionally promotes age-associated diseases like type II diabetes and atherosclerosis, thereby reducing health and wellbeing and causing premature ageing. Increasing evidence suggests that infection with parasitic worms can protect against this and indeed, our studies suggest that the worm product ES-62 can correct some of the inflammatory and metabolic faults associated with such unhealthy ageing. We are therefore trying to work out how this happens as by identifying the mechanisms involved, we can design drugs that stop or even reverse this process and hence promote health and extend life.

   3.3 Start Date: 01/06/17  Finish Date: 12/07/17

3.4 Original project aims and objectives (56):

Our core aim is to investigate whether ES-62-mediated promotion of health- and lifespan in HFD-fed mice reflects direct and/or indirect effects on the gut microbiota. Specifically, it is planned to determine whether ES-62 modulates

- the levels of bacteria implicated in HFD-induced dysbiosis
- gut pathology in HFD-mice
- inflammatory cell infiltration of the gut and/or their functional responses

3.5 Methodology: Summarise and include reference to training received in research methods etc. (249):

The project, initially aimed at studying the effects of ES-62 in HFD-fed mice, was redefined with the goal of studying ES-62 in arthritic mice instead. This decision was made based on sample availability and fitness of the project inside the lab.

Analysis focused on archived tissue samples from mice undergoing inflammatory arthritis. The subject pool comprised: healthy mice ("Naive"; n=4), mice with collagen-induced arthritis (CIA; n=8) and mice with ES-62-treated arthritis (ES-62; n=8). Replicate groups were treated with antibiotics (Abx; Vancomycin, Neomycin and Metronidazole) before (day -7) and during development of arthritis (cull=day 33), in order to observe the effects of disruption of the microbiota. Arthritis was assessed by articular score where naive ±Abx = 0; CIA = 4.75 ±1.3; ES-62 = 0; CIA +Abx = 2 ± 1.04 and ES-62 +Abx = 3.125 ±1.29.

Levels of total bacteria and particular genera (Bacterioides (Bac), Lactobacillus (lac), Segmented Filamentous Bacteria (SFB)) in tissue from ileum, colon and spleen were analysed by qPCR of TRIzol-extracted 16S rDNA, using SybrGreen-labelled primers. Likewise, mRNA was reverse transcribed into complementary DNA (cDNA) in order to assess expression of inflammatory mediator genes (relative to $\beta$-actin) via qRT-PCR using SybrGreen primers or Taqman probes. CT values from the qPCRs were transformed into fold change values (fc) for the targets and $\Delta$CT values for the calibrators ("all bacteria" and "$\beta$-actin").

To assess gut pathology, sections and "rolls" of gut tissue (ileum and colon) were stained with Alcian blue (mucin production) and haematoxylin and eosin (H&E, cell infiltration).

3.6 Results (figures in appendix) (211):

Mice with arthritis (CIA, CIA+Abx, ES-62+Abx) generally tended to show (Figs. 1-3) decreased levels of Bacteroides and lactobacillus populations and higher levels of SFB, data consistent with the latter being reported to be associated with disease. In addition, reflecting the association of decreased bacterial diversity with arthritis, the levels of total bacteria were reduced (higher CT values). On the contrary, ES-62-treated mice showed values more similar to those of the Naïve group, (higher levels of Bacteroides and Lactobacilli and reduction of SFB, most clearly seen in Fig. 2).
In terms of inflammatory mediators, higher levels of expression of IL-17R, IL-1B and HMOX in colon were seen in the groups exhibiting arthritis while these levels are lower in ES-62

(Fig. 4). Indeed, in some cases the levels of expression of these are higher for ES-62+Abx than they are for CIA or CIA+Abx.

Reflecting the increased expression of IL-17R, IL-1B and HMOX in arthritic groups, the gut tissue from such mice exhibited higher levels of inflammatory cell infiltration and tissue damage relative to that observed in Naïve or ES-62-treated mice. This is particularly evident when comparing colon sections from Naïve and ES-62 mice with those from CIA and CIA+Abx mice (fig. 5) or ileum sections from ES-62 versus and ES-62+Abx mice (fig. 6).

3.7 Discussion (431):

The qPCR analysis of bacterial DNA (fig. 1-3) show several patterns relevant to our objectives as, consistent with reports that bacterial abundance and diversity is reduced in inflammatory disease, the total levels of bacteria in the colon and ileum are lower (higher $\Delta$CT values) in CIA mice relative to the other groups and it appears that ES-62-treatment helps preserve, to some extent, a normal gut flora. Reflecting this, levels of "beneficial" Bacteroides and Lactobacillus species are higher in Naïve and ES-62 and lower in CIA (+Abx; apart from one outlier) groups. One exception to this is the abnormally high value for lac in CIA+Abx ileum. By contrast, levels of SFB, which has been associated with arthritogenesis in mice, are increased in mice with arthritis relative to healthy and ES-62-treated mice. Therefore, the microbiome in ES-62-treated individuals is more similar to that of healthy individuals than it is for mice with arthritis.

These changes in the microbiota were reflected by changes in inflammatory mediators. For example, we can see how expression of pro-inflammatory genes is high in the ES-62+Abx and CIA groups which exhibit strong arthritis (Fig. 4). This suggests that Abx-perturbation of the gut microbiota may affect ES-62-mediated protection as was generally observed in all of the three organs that were studied (ileum, colon and spleen). In addition, where several CIA individuals have been tested, the range in results is rather broader than it is for other groups. This has led us to think that this variability may correlate with the degree of arthritis and this will be followed up.

Finally, more tissue damage was apparent in CIA and also in ES-62+Abx samples, reinforcing the hypothesis of the microbiota playing a crucial role in promoting arthritis. In particular, whilst villi are clearly visible in gut tissue from Naïve and ES-62 mice, in CIA ±Abx mice the gut has been inflamed to the point that these structures are no longer noticeable (Fig. 5). The walls are much thicker in the CIA+Abx specimen, and immune cell infiltration can be seen on the left side of the CIA gut photograph. Likewise (Fig. 6), the difference between ES-62 and ES-62+Abx tissue is clear, with that from the ES-62 group looking like that of the healthy, Naïve guts: by contrast, tissue from ES-62+Abx shows no recognisable villi and is profoundly damaged and swollen.

This would all suggest that whilst ES-62 does indeed regulate CIA-related inflammatory processes, it requires normal host flora to act effectively. The next steps now would be to identify the mechanisms through which ES-62 works, and how a healthy microbiota enables its action.

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

   This studentship has not only helped me improve my knowledge on science, but it has also (and, in my opinion, more importantly) showed me what it is like to work in a lab, doing research alongside other people while being part of a team.

   I have been able to learn very valuable techniques to the point that I was able to work on my own and obtain good quality results from scratch which, beyond just contributing to my practical skills, was also a great way to gain confidence in one self and pride for one's own work.

   Opportunities like this are determining for us students, who are still in the process of making our career choices. I have been reassured that taking part in research is my main goal after finishing my degree, and I have also been provided with priceless experience and background that will doubtlessly help me achieve it.

   I am truly grateful that the Head of College Scholars list offered me this chance to learn and improve, and also that Prof Margaret Harnett and her team allowed me to work with them and taught me so much despite my inexperience. I have left the lab with new encouragement to keep pursuing my goals in my studies and in life.

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

   The Harnett team is engaged in raising public awareness via their websites (es62.webstarts.com; Twitter @HarnettLabs and Bugs from Drugs Facebook page) as well as through their outreach activities in the community, such as schools visits. The studies performed in this placement will form the basis and inform on the direction of future studies that will be discussed in such forums and will eventually lead to publications in quality biomedical journals. In addition, I was able to disseminate my data by presentation a work in progress to the Harnett and Pineda research groups within the Institute of Infection, Immunity and Inflammation.

6. Signatures:

| Supervisor | Date | Student | Date |
|---|---|---|---|
| Margaret Harnett | 31.07.17. | | 01-08-17 |