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: McMurray Forename: Gregor

   E-mail address: 1105266M@student.gla.ac.uk

2. Supervisor:

   Surname: McSharry Forename: Charles

   E-mail address: Charles.McSharry@Glasgow.gla.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   Matching serum IL-33 and IL-18 concentrations as a functional counterpart to gene SNPs in severe asthma.

   3.2 Project Lay Summary (copied from application):

   Several hundred Glasgow patients donated blood for a UK-wide genetic study to investigate mechanisms of severe asthma. This identified the involvement of genes controlling lung inflammation. These genes are called IL33 and IL18. We will measure the amounts of IL33 and IL18 in the blood samples of asthma and healthy patients to see if they confirm this finding. Cigarette smoking is a major determinant of severe asthma

therefore we will compare IL33 and IL18 with the patients' smoking history to see if there is a link. If so then these protein may provide new insight into possible mechanisms of severe asthma that is characteristically refractory to conventional treatment.

3.3 Start Date: 22/07/13 Finish Date: 20/09/13

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

We aim to analyse previously measured serum IL-33 concentration to establish whether the levels correlate to disease state and severity or smoking status. We also aim to quantify serum IL-18 in smoking and non-smoking healthy, asthmatic and patients with chronic obstructive airways disease (COPD) as a pathological control group. We will compare the serum IL-18 levels with disease state and severity to establish if there is a correlation. We also aim to find out if smoking status affects serum IL-18 levels.

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

IL-18 levels of 353 serum samples from smoking and never-smoking healthy controls and patients with severe, moderate and mild asthma and COPD were measured using an enzyme-linked immunoassay (ELISA) method (kit from MBL).

Training: training in lab healthy and safety, how to identify a clinical research need and establish a hypothesis, importance of ethics, basic scientific principle and planning of experiments.

Technical: my optimising the IL-18 assay conditions and quality control provided a blueprint for understanding laboratory assays.

Analysis: Data was recorded on spreadsheets and statistical analysis was performed using Minitab software (Minitab Inc., State College, PA, USA). IL-33 levels were compared with lung function and other asthma biomarkers by correlation analysis. The IL-18 levels were compared with clinical categories and smoking history. Statistical

training: Principles of statistical analysis were explained including difference between Parametric and Non-parametric analysis. This was relevant when comparing skewed IL-18 concentrations between healthy controls, asthmatics and COPD patients (Kruskal-Wallis and Mann-Whitney tests). A p-value <0.05 was considered significant.

Overall training: this studentship gave a snap-shot of how a clinical department (Respiratory Medicine Gartnavel Hospital) integrated with a basic research facility (Glasgow Biomedical Research Centre) to exchange ideas, develop research question, how to plan, execute, interpret and report this. This was highly relevant because I am interested in a career in academic medicine related to inflammatory disease and this gave me an opportunity to engage closely with clinical scientists and gave me an insight behind the scenes in an immunology lab.

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

<u>IL-33 Serum Levels and Disease State</u>

The severity of asthma was measured using Global initiative for asthma (GINA) guidelines. No correlation was found between IL-33 levels and the severity of disease (p = 0.937) (Table 1). However, there was an association between IL-33 levels and cigarette smoking with smoking reducing IL-33 levels (median: smokers 203pg/ml vs. non-smokers 1,548pg/ml) (p = <0.001) (Table 2). IL-33 concentrations correlated with pack history (r = -0.196, p = 0.006) but not with the number of cigarettes smoked per day (r -0.083, p = 0.514). IL-33 concentration is not significantly different between patients on oral steroids and those not (p = 0.613). No correlation was found between IL-33 concentrations and the asthma biomarkers of lung function (FEV1% predicted normal), reversibility by β-agonist, exhaled nitric oxide concentration, asthma control score, serum IgE, blood eosinophil count and BMI (Table 3). There was also no difference in IL-33 between atopic patients and

non-atopic patients (median: atopic 621.8pg/ml vs. non-atopic 654pg/ml) (p = 0.719).

IL-18 Serum Levels and Disease State

IL-18 levels were significantly reduced in asthmatics and COPD patients compared to healthy controls (p = 0.008 and p = 0.002) with the reduction more pronounced in COPD patients than asthmatic patients (Table 4a, table 4b and Fig. 1). However, no significant difference was found between IL-18 levels and the severity of asthma or COPD (p > 0.05). There was also no correlation between IL-18 levels and smoking status (Table 5). A correlation was found between IL-18 levels in the first sample and IL-18 levels in the second sample, roughly one month later (r = 0.830, p = <0.001) (Fig 2).

3.7 Discussion (500 words max):

IL-33 and IL-18 are both members of the IL-1 cytokine family with pro-inflammatory and immune-regulatory functions. IL-33 binds to ST-2 receptor to initiate type 2 immune/inflammatory (T_H2) responses. T_H2 responses are considered to be "allergic responses" and this is observed in asthma. IL-18 is a potent inducer of IFN-γ release which is a characteristic cytokine of a type 1 immune/inflammatory (T_H1) response. A T_H1 response is thought to push inflammation away from the "allergic" root.

Single nucleotide polymorphisms (SNPs) in the IL-33 and IL-18R genes have been linked to severe asthma but the biological significance is unknown. The IL-33 concentration was unrelated to asthma severity, nor affected by steroid treatment and was unrelated to a variety of asthma biomarkers. IL-33 was strongly affected by cigarette smoke with smokers having significantly reduced concentrations. This was the opposite to what was expected since smoking is known to make asthma worse so it was thought that it may increase pro-T_H2 IL-33 concentrations. This suggests that the relationship between IL-33

and asthma severity and biomarkers should be re-calculated separately according to smoking status. The observations suggest that asthma among smokers may have a different pathogenic mechanism and should be taken into consideration when interpreting SNP analysis.

IL-18 can be identified and quantified in serum samples. There was a wide concentration range which seemed to be stable since the values were replicated in a second blood sample taken at around one month after the first blood samples. This suggests that IL-18 expression and serum levels are phenotypic and will therefore be relatively consistent throughout life. This is a contrast to other pro-inflammatory cytokines (e.g. TNF-α and IL-6) which increase in response to infection/injury but then decrease once the infection/insult is cleared. Serum IL-18 was significantly reduced in patients with asthma and COPD compared to healthy subjects and was unaffected by cigarette smoking. This may suggests that individuals with low IL-18 phenotypes are more susceptible to developing asthma since low IL-18 will result in low IFN-γ production and therefore a push towards the "allergic" T_H2 response. It is therefore of interest to compare IL-18 phenotypes with disease state.

IL-18 has been found to promote both T_H1 and T_H2 responses depending on the surrounding cytokine environment so it may be that changes in the IL-33 concentration through smoking may cause IL-18 to promote T_H2 responses as opposed to T_H1 responses. It would be of interest to measure IL-18 and IL-33 levels to establish if there is a correlation between the two cytokines and compare this to disease status/severity. We have identified a clinical effect on IL-18 and a smoking effect on IL-33 that might help to clarify the potential role of these cytokines in the pathogenesis of airway disease. It is of future interest to measure other members of the IL-1 family to establish if they correlate to disease state/severity. If so then drugs to either block or enhance

the action of these cytokines may provide a new treatment option for severe, steroid refractory asthma.

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

The studentship has been valuable as it provided insight into how research, especially lab work, operates. It was interesting to see how a project starts from an initial idea/hypothesis and then develops into practical experiments to generate data which can then be analysed to ultimately produce a publishable paper. One of the most important things I learned from the studentship was the importance of being organised and planning, especially in terms of lab work. I learned that proper planning of the experiment including volumes and dilutions of solutions required is very important otherwise there might not be enough of a solution left or it may be used in the wrong dilution which will affect the results. I also learned that experiments don't always work initially (especially if a new kit is being used) and not to get frustrated about it. If the experiment didn't work I learned to go back to the beginning and think about what might have been the cause and adapt accordingly.

I also realised the importance of statistical analysis of data as it allows conclusions to be drawn from hundreds of meaningless numbers. I also learned the importance of taking care when entering raw data as this could potentially be a large source of error and alter the final conclusions.

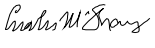
Overall, the studentship has made be appreciate just how much time and effort goes into producing one research project from start to finish. I also appreciated that once one project is completed it leaves several unanswered questions, each of which may be an individual project in its own right. I realised that each project is only one part of the bigger picture and that cooperation with other teams is essential to integrate the little pieces in order to solve complex problems.

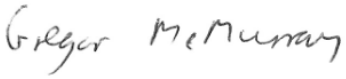
5. Dissemination: (note any presentations/publications submitted/planned from the work):

I gave an informal presentation of this work to my supervisor.

The IL33 and IL18 data and their relationship to asthma and to smoking is important because asthma patients who smoke do not respond well to conventional steroid therapy and new understanding of the inflammatory process is required in order to prompt studies for new therapeutics. These data suggests that smokers with asthma have a different inflammatory process and this will be prepared for publication in due course and the financial support of the College will be acknowledged.

6. Signatures:

Supervisor  Date 20/09/13

Student  Date 20/09/13