Home Office

NON-TECHNICAL SUMMARY

Immune control of parasite infection and tissue injury

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

parasites, Tissue repair, Infection, Allergy, Scarring

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult
Gerbils	Adult
Rats	Adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The 'type 2' arm of the immune system is important for fighting infection with parasitic worms but is also involved in reducing inflammation and repairing damaged tissues. The aim of this project is to understand how ‘type 2’ immunity performs these different but related tasks.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Type 2 immunity is essential to maintain tissue health in the face of infection with large tissue-migrating parasitic worms. Theses immune pathways are needed both to repair the damage caused by these worms and to reduce parasite numbers. Type 2 immunity is also involved in tissue repair more generally influencing how our bodies produce and lay down the molecules outside our cells that give our bodies structure, elasticity and hydration (the extra-cellular matrix). However, when these pathways become dysregulated they can lead to allergy, chronic diseases and tissue scarring. Learning about these immune pathways is relevant to understanding many non-parasitic diseases that involve the type 2 immune response such as asthma.

What outputs do you think you will see at the end of this project?

Outputs will include:

1. A greater understanding of how tissue repair pathways interact with the immune system to cause or prevent disease, and critically how this immune information is communicated between organs in the body.

2. Publications disseminating our findings to global scientists.

3. Increased collaboration with clinicians on the use of drugs that block type 2 immunity

Who or what will benefit from these outputs, and how?

Short term benefits will be to other medical researchers who study parasitic worm infection and wound repair. Within the five years of the project, clinicians currently using drugs that block type 2 immunity could have a better understanding of their potential use and limitations. We also anticipate a greater understanding of the immunology of the body cavities that surround our intestines and lungs.

Mid to long term benefits will be to enhance our understanding of existing treatments for allergic diseases. These include drugs such as dupilumab, which is directly relevant to this project and is increasingly used in the clinic. Our work may lead to broader potential uses for these drugs, or a

greater understanding of their potential limitations. Our work will also contribute to the development of new potential therapies designed to target the extra-cellular matrix. Our work to study how the immune system communicates between organs in our body has the potential to enhance understanding of why some people have disease symptoms in more than one tissue. For example, we may discover pathways that link allergic skin disease to lung diseases such as asthma. We also anticipate helping the development of ways to prevent or treat diseases of the body cavity, such as scarring that follows abdominal surgery, or how cancer cells can use these cavities to move around the body.

How will you look to maximise the outputs of this work?

To ensure maximum dissemination, we aim to publish in journals that reach a wide, and if possible, interdisciplinary audience. Furthermore, we place the first drafts of our published data on open access repositories such as www.biorxiv.org. To prevent unnecessary repetition of experiments by others, we seek to publish all data generated under this project including negative results.

Communication will also be by presentation at local, national and international congresses, where we regularly present unpublished data. We also talk regularly to clinicians at these meetings. In addition, our recent funding includes co-investigators who are clinicians. To enable rapid translation of our findings to the clinic we will exploit new and existing collaborations taking advantage of the University support toward translation. Importantly, all our studies are highly collaborative ensuring communication across different research groups.

We also actively engage with the public, in particular, we have media training and talk to the media (e.g. radio interview) about recent cases of parasitic infection in the news.

Species and numbers of animals expected to be used

- Mice: 25,000
- Rats: 240
- Gerbils: 120

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are the most versatile and tractable model for establishing models of immune pathology and extracellular matrix remodelling. The immune system and matrix components have been extensively described in mice and continue to provide insight into their importance during asthma pathology, viral and parasite infections in humans. Furthermore, the availability of immunological/matrix reagents and transgenic lines for mice, allows for approaches to manipulate immune-matrix components that delineate multi-factorial cell-cell and cell-matrix interactions that would not be possible using other species.

We will generally use adult mice in our experiments (>6-8 weeks of age), as at this age the mouse's immune system has developed to a degree that models the adult human immune system. However, important data suggest that modulation of the immune system in early life can alter susceptibility to external insults such as infection. Therefore, in some experiments we will use neonatal or juvenile mice to try and uncover important ways in which alterations in the immune system during early life can have important effects on the immune system.

Gerbils are the only suitable laboratory rodent capable of generating sufficient numbers of the blood circulating stage to allow transmission to the mite vector. Gerbils are thus essential for lifecycle maintenance, but do not have the genetic or immunological tools needed for detailed research. Mouse pups (typically 6-10 days old) are needed to maintain the mite vector, which transmits infective larval stage of the filarial parasite. Rats are required to generate infective larvae of the hookworm parasite we study.

Typically, what will be done to an animal used in your project?

Typically, mice will be infected with parasites, viruses, and/or be exposed to an allergen, and/or receive a single or multiple injections containing immunomodulatory substances (e.g. antibodies to neutralise a specific immune mediator or deplete a specific cell type, or cells to promote a particular response).

Experiments might look at the immediate immune response in the first few days after infection, allergen exposure, or administration of an immunomodulatory substance, or may last several months to allow full response development, or assessment of immune memory. These experiments will typically last between 1 day and 3 months. For longer experiments, mice may receive multiple doses of an immunomodulatory substance (e.g. once a week for 2-3 weeks), and samples (e.g. blood) may be taken to monitor immune response development over time. Experiments will end with animals being killed humanely, sometimes under terminal anaesthesia.

The cumulative experience of mice will typically be exposure to 2 or 3 procedures that may each cause short but usually separated periods of typically mild or potentially moderate degrees of suffering.

Separate from the above experiments, some genetically altered animals will be used only to breed and maintain animal lines.

Gerbils are typically infected with parasites, and then after several weeks (when parasites are producing offspring), they will be exposed to mites. The exposure to mites will typically occur 3-5 times and typically 2 weeks a part.

Rats are typically infected with parasites and kept for 8 days while they are generating eggs in their faeces.

What are the expected impacts and/or adverse effects for the animals during your project?

Most of the animals undergoing experimental procedures, even with immunological manipulation, will experience mild, and no more than moderate, severity limits.

Breeding and maintenance of genetically altered mice with specific deletions in immune function genes or transgenic expression of immune receptors are not expected to exhibit any harmful phenotype. Our assessment of damage and repair due to parasite migration, viral infection or allergen challenge can result in local or systemic inflammation and pathology. This can manifest as weight loss, the involuntary bristling of fur, reduced spontaneous activity and reduced response to external stimuli. However, in most cases, only a small proportion of experimental animals will develop beyond mild symptoms to moderate severity limits. In investigating lung inflammation by airway manipulation using established models, some animals (5-10%) may experience temporary (less than 24 hours) respiratory symptoms resulting in moderate severity limits. However, most experimental animals will not develop beyond mild symptoms. Some protocols will involve general procedures such as restraint, injection or use of anaesthesia. All of these provide the possibility of adverse effects, but none beyond moderate severity. All animals will be humanely killed at the end of each experiment.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

This project works to a maximum severity of moderate. Across the license, we expect the majority of mice, rats and gerbils undergoing experimental procedures to experience mild (approximately 85%) severity, with <15 % experiencing moderate severity. Animals in breeding protocols should all experience subthreshold.

What will happen to animals used in this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We are studying complex processes that develop over time and trying to understand how the immune system handles these conditions and how different tissues communicate across the body to ensure that the right response occurs in the right place. All of these models involve the entire body, from the parasite infection model that involves migration of the worm from the skin to lung, to the viral infections which have systemic consequences, to the allergic airway remodelling that requires chronic exposure, and thus cannot be replicated in vitro. While experimental human studies of infection are sometimes preformed in very controlled settings, it is not possible to analyse the whole-body response in the different tissues. Similarly, the parasites that we study cannot be maintained in vitro and need a mammalian host to develop from the infective larval stage through to mature adulthood to mate and produce more parasites.

Which non-animal alternatives did you consider for use in this project?

Where it is appropriate we use in vitro systems to address particular problems. For example, we add immune factors to cell lines, to study the response in vitro. We have also worked with colleagues to add macrophages (a major immune cell of interest) to tissue organoids to understand the interaction of these cells with the tissue structure. Increasingly, we are using human tissue, for example from lung biopsies to assess the extra cellular matrix in the lungs of asthmatic vs non-asthmatics. Importantly, with more advanced technologies, particularly in computing, we are able to be move selective in the immune pathways we study, ensuring that what we are doing is the most relevant to human disease.

Why were they not suitable?

Our studies rely on looking at the immune response to infection or other challenges in the context of the whole body. We consider the consequences of exposure in both the local tissue (e.g. skin) and the impact in other distant tissues (e.g. lung). Many of our models involve long term infections, in which parasites migrate through the body and mature, with each parasite stage interacting with the host in distinct ways. Our allergy models look at the consequences of repeated exposure over time. Many of our studies consider the long term effects of exposure to allergens/parasites in the immune system later in life. None of these processes can be tested in vitro or with organoids. We are particularly interested in how immune cells interact with the extracellular matrix, which changes over time and in reponse to inflammation, a process that itself is poorly understood and cannot be replicated in vitro. We have also found that when we remove our immune cells of interest from the body, they lose many of their tissue-specific features, making the use of cell lines impractical.

Experiments to track immune cell interaction, activation and function over time are not possible with human samples, even where we have access to tissue, and we cannot experimentally manipulate humans.

Our research also depends on generating the infective stages of nematode parasites. There is no alternative means of generating this stage other than in a mammalian host (mice, rats or gerbils).

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of mice has been estimated based on experience gained under my previous Home Office licenses, taking into account breeding strategies for genetically altered mice, and anticipated numbers of planned studies over the course of the license.

Reflecting Replacement, Reduction and Refinement of our protocols over the past 5 years, we have reduced our anticipated numbers of animals for this application by around 40% from our previous project license.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We now purchase only the number of gerbils that we need from a commercial supplier, with a reduction in overall gerbil numbers compared to breeding in house.

Time mated female mice are bought in to supply pups for the mite feeds. The number of pups has been reduced to 9 per cage to ensure the pups are the correct size to use in the mite feeds. Surplus pups are not killed but fostered onto females from which pups have been taken from previously. Some of these pups are used when they come of size. The animal facility also make use of the foster mums for any pups from other parts of the animal facility.

For all of our mouse experiments, in-bred mice are used to reduce experimental variation, which makes it possible to use fewer animals to achieve statistical significance. For the majority of our studies, mice from the same litters are used for control and experimental mice, reducing variation that can occur due to differences in the microbiota. Overall, our experiments are designed to reduce the number of variables (for example age) to as few as possible and thereby reduce the number of control groups required.

All lab members are introduced to the NC3Rs experimental design assistant and encouraged to use it. However, everyone in the lab is trained in statistical methods and these are regularly discussed at lab meeting. We sometimes use more rigid criteria than the NC3Rs assistant if all agree that it the most appropriate for the experiment in question. Randomisation and blinding are included whenever practically possible. Tissue-sharing is a major tool we use to reduce animal usage.

A significant proportion of our animal use is related to breeding programmes for genetically altered lines. We follow the advice of our animal facility staff to optimise breeding, and regularly discuss numbers at lab meeting to ensure we do not overbreed. Where possible and appropriate, we use substances that can target or block immune processes in wild type mice, to reduce use of genetically altered mice.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We actively discuss all experiments in advance with all members of the lab so as to avoid any duplication and allow lab members to share animals. Tissue sharing occurs not only within our own lab, by through active discussion with other research teams, where we have found opportunities to work together.

The increased use of genetically altered animals has led to more complicated breeding strategies and, as a result, larger colonies. We reduce the numbers of these animals in our experiments by using littermates as controls wherever possible. Indeed, by including the heterozygotes in our studies, we have made important discoveries on the impact of gene dose. Additionally, when a particular strain is

not being used experimentally we work closely with the animal technicians to develop a breeding strategy that maintains low numbers of stock animals.

We also regularly monitor animal numbers at lab meeting to ensure they are being used when needed – and all lab members are aware of any available mice.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Litomosoides sigmodontis is a mouse model for human filarial disease, reflecting closely the life cycle timings in humans. By using different strains of mice we can model both susceptibility and resistance to disease, as well as key features of immune regulation. A limitation of this model is that it does not reflect pathology to the disease, but the result is that we can study immune responses and parasite control with minimal animal suffering. In addition, this is the only model of type 2 immunity in the fluid-filled cavity that surrounds the lung, a tissue that is very understudied but relevant to multiple human diseases.

Nippostrongylus brasiliensis provides an excellent model of tissue repair in the lung. The migration of the parasite through the lung induces a classic inflammatory response, followed by an appropriate type 2-mediated repair response. This allows us to study injury repair pathways and by adjusting the dose we are able to minimise animal suffering but still study the pathways of immune cells and the remodelling of tissue structures that lead to effective repair. We compare these to models where repair is less efficient such as following flu infection, or following chronic allergen exposure. *H. polygyrus* and *T. muris* are natural parasites of mice that have a direct fecal-oral route and allow us to study parasites that only infect that gastrointestinal tract and which cause minimal adverse effects.

Mice represent the most appropriate species for in vivo study of immunity, because of the extensive knowledge of their physiology as it relates to humans, the genetic and biological tools available and the ability to be easily bred and handled. We will only use mouse models that have been refined to minimise animal distress. Procedures involve administration of virus or allergens by inhalation, or parasites by injection or direct delivery, and injection of immune modulators to reduce or alter disease progression.

Most of the animals undergoing experimental procedures, even with immunological manipulation, will experience mild, and no more than moderate, severity limits. Doses and timing are carefully managed such that animals will experience minimal suffering. We are constantly assessing and refining our approaches to ensure robust experimental results whilst minimising pain, suffering or distress.

We have recently acquired genetically modified mice that do not necessitate us to perform whole-body irradiation for the study of certain genes.

Why can’t you use animals that are less sentient?

Standard laboratory mouse strains are readily infected with the pathogens or allergens we study via the natural route (e.g. via the nose). The route of infection, site of pathogen development and the immune cell response are similar to that in man. Unfortunately, none of this can be replicated in less sentient species. To our knowledge, no other species of lesser sentience can fulfil the requirements of this project to the same extent as the laboratory mouse. We are studying long and complex immune processes and trying to understand how different different parts of the immune system communicate with the body to orchestrate an appropriate response. For example, we need to understand how cells communicate with the extra-cellular matrix, a scaffolding made of large proteins and other molecules which provides structure, holds cells together, and helps them communicate. The extra-cellular matrix components that regulate immunity are highly conserved between mouse and man, but would be very different in a non-mammalian species. We cannot use terminally anaesthetised animals as we require the mice to develop immune responses to the external challenges, and to analyse the outcomes of these challenges over time and in response to interventions.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

All procedures will be performed by trained and skilled personal licence holders, who will handle animals with care. Animals will be monitored for adverse effects using score sheets previously developed in conjunction with the NVS and NACWO. These score sheets have proven to allow for objective measurements of clinical signs associated with adverse effects to determine when humane endpoints have been reached.

In line with the establishment's policy, we will adopt the latest techniques in animal handling (e.g. cupping) to significantly reduce the stress associated with procedures. Furthermore, where possible, the least invasive methods for dosing and sampling will be applied.

Anaesthesia and analgesia will be provided where suitable (e.g. for humane restraint, during or in recovery from surgery). Antibiotic cover will be used to prevent opportunistic infections after irradiation.

We are constantly considering potential refinements to improve animal welfare including enrichment. For example, gerbils are prone to seizures when stressed. Our team began to reward them with sunflower seeds each time they are handled for a cage change or blood sample. The seizures are now very rare, and gerbils exhibit positive behaviour when the technician approaches in anticipation of the seeds.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

The government animal testing and research: guidance for the regulated community (https://www.gov.uk/guidance/research-and-testing-using-animals)

Morton et al 2001, Refining procedures for the administration of substances; Laboratory Animals, 35, 1-41

The NC3Rs webpage: https://www.nc3rs.org.uk/

The PREPARE and ARRIVE guidelines: https://arriveguidelines.org, https://norecopa.no/PREPARE

Standard Operating Procedures developed with the animal facility and the named veterinary surgeon.

For specific models, we read papers from other groups doing similar experiments, as well as consulting directly with other researchers to discuss the most refined procedures.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our team consult closely with the animal facility and take full advantage of the extensive resources provided to ensure we are following current best practices. We will continue to work closely with our local 3Rs representative to ensure we stay informed about the advances in the 3Rs. For example, we attend Experimental Design workshops to ensure we achieve our scientific aims with the minimal number of mice.