Home Office

NON-TECHNICAL SUMMARY

Type 2 immunity in infection and maintenance of tissue health

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

No answer provided

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant, embryo
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?

We want to understand how the immune system functions to protect the host from parasitic worms, which cause damage as the migrate through the body. By extension, we aim to learn how these immune pathways help heal wounds or cause disease by overzealous tissue repair.

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?

Helminths (parasitic worms) infect over a quarter of the human population, and an even greater proportion of animals. Although not generally fatal, they cause substantial suffering, particularly in low income regions. Disease can be caused by competition for nutrients, physical injury due to migration through the body, and/or an excessive immune response to the parasite. The type 2 arm of the immune system is a highly complex network of cells and molecules, which is very distinct from the pathways that control infection with bacteria and other microbes. Type 2 immunity is necessary both to control helminth numbers and to repair the physical damage they do. However, overzealous type 2 responses can lead to allergic diseases, asthma and chronic tissue scarring (fibrosis). Wound repair and scarring features of type 2 immunity are common even in regions with no helminth infections. Thus, fundamental research into the mechanisms and consequences of the type 2 immune response will lead not only to improved understanding and control of helminth infection, but greater understanding of the many allergic and fibrotic diseases that are major killers worldwide.

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

This project aims to disseminate new knowledge by publication in peer reviewed journals and presentations at conferences, seminars and workshops. We hope that in the longer-term our work will contribute to new immunology-based therapies. In the 5 years, we aim to continue our high standard of publication, averaging more than 5 research papers per year in highly-respected peer-reviewed journals.

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

This project aims to answer fundamental scientific questions. The new knowledge generated, and the unravelling of important immune mechanisms, will be relevant to a broad range of human and animal conditions.

Parasitic worms (helminths) afflict over 1/4 of the human population and the vast majority of wild mammals. They also represent an enormous economic burden in terms of livestock productivity. In the next five years we expect to make significant strides in understanding the mechanisms by which these parasites are killed, which will be relevant in the longer-term to prevention or treatment strategies. In

addition, helminth infection can increase susceptibility to other infectious agents, such as viruses and bacteria. Our analysis of how cells function during helminth infection should help us understand how to manage these common co-infections.

Our work is directly relevant to fibrosis (tissue scarring) and asthma. Fibrosis in people is estimated to contribute to 45% of all deaths in developed countries. In the UK, around 5.4 million people are currently receiving treatment for asthma, which equals 1 in every 12 adults and 1 in every 11 children. We expect our work over the next 5 years to define the key molecules involved in how the tissue becomes restructured and remodelled to cause more severe disease, and potentially whether the process can be reversed once it has started. This is particularly relevant as new biological drugs that target type 2 immune pathways are now used in the clinic for skin disorders, and our studies would highlight their potential role in other diseases.

Because several of our models involve the investigation of the body cavities, they may provide important insight into a major clinical problem: the adhesions (inner body scars) that follow abdominal surgery. The underlying cause of these very painful adhesions are poorly understood, but have been linked to the immune cells we study. Our unique expertise in the cavity around the lung (the pleural cavity) will also provide insight into build-up of fluid in the cavity resulting from heart failure, pneumonia and cancer. In particular, we are interested in the possibility that idiopathic pulmonary fibrosis, a fatal lung disease of unknown origin, may start in the pleural cavity. Our work with a parasite that lives in the pleural cavity, has the potential to reveal whether scar tissue initiated in the pleural cavity can move into the lung.

One of the most specific outcomes we hope to achieve is an understanding of the function of chitinase-like proteins. Increased levels of these proteins in the blood are markers of poor outcome in a wide variety of diseases from asthma to cancer but their function is unknown. We hope to reveal why chitinase-like proteins are associated with disease and whether drugs designed to block them could be beneficial. This work could lead to new therapeutics in the next 10 to 15 years.

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

Communication of our findings will be primarily through publication in widely-read peer-reviewed journals, but also presentation at local, national and international congresses and institute seminars. To ensure maximum dissemination, only journals that allow open access without payment by the reader will be considered. Furthermore, we will place the first drafts of our published data on an open access repository such as www.biorxiv.org. To prevent unnecessary repetition of experiments by others, we will seek to publish all data generated under this project including negative results.

To enable rapid translation of our findings to the clinic we will exploit new and existing collaborations with local clinicians as part of the translational environment within our institution. We have highly effective systems in place for technology transfer. Additionally, pharmaceutical and biotech companies could be engaged through presentation at national and international forums at which representatives are often present.

Species and numbers of animals expected to be used

- Gerbils: 500
- Mice: 40000
- Rats: 250

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.

We study adult mice because the immune system, tissue organisation and development of all mammals are similar allowing mice to be a model for humans and other animals. We also use mice because scientists have created many genetically altered mouse lines that allow us to dissect in fine detail what happens during immune responses. Genetically altered mice and many of the tools designed to work with mice allow us to define in precise detail how particular cells and particular molecules work together to repair tissue or fight infection. By manipulating these cells and molecules, we can understand how these processes go wrong during diseases such as asthma.

We also use gerbils and rats to maintain our parasite lifecycles, and neonatal mice to maintain the mite vector that transmit parasites. In each case, the animal is the most susceptible to infection, allowing us to use the least numbers of animals to maintain the parasites or mites in sufficient numbers for experiments.

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

Typically a mouse will be either 1) infected with a parasitic worm by injection under the skin, 2) injected with a molecule that induces a scarring response, or 3) given a mixture of allergy inducing molecules through the nose. Experiments might look at the immediate immune response in the first few days but can last as long as 3 months to allow full development of the parasite, or full development of diseased tissue (e.g. asthmatic lung).

Many animals will also receive an injection of a molecule or cells to modify the immune system. For example, an animal might receive an antibody that will get rid of a particular immune cell. In addition, small volumes of blood may be taken from a vein, for example to screen for parasites or blood cell changes. Experiments will often end with animals being killed under terminal anaesthesia.

Hence, 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.

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

The vast majority of animals will experience no adverse effects or only mild adverse effects. The parasite infections are generally well tolerated and will rarely reach moderate severity. In the hookworm model a heavy infection can cause weight loss and laboured breathing but we use low doses to avoid this. The fibrosis and allergy models are designed to assess the structural changes to the tissues without major overt clinical symptoms. However, some manipulations can make animals more susceptible to infection, injury or allergy, which may increase the severity from mild to moderate. All animals will be humanely killed before they exceed moderate severity limits.

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)?

Based on our previous experience, we expect approximately 85% of mice to experience mild severity and less than 4% to experience moderate severity. The gerbils and rats all experience only mild severity.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

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?

The immune system relies upon a complex series of interactions, which occur between immune cells, structural cells and the extracellular matrix. The nematode models involve migration of parasites through living tissue. To understand such complexity, it is essential to undertake research in vivo, since this cannot yet be modelled meaningfully in vitro.

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

1) Analysis of human biopsies.

2) Use of cell lines

3) Use of organoids - artificially grown cells that resemble an organ.

Why were they not suitable?

The types of experiments required to track cell function in vivo are not possible with human tissue biopsies, nor can we experimentally infect humans.

Many location-specific features of cells are lost once they are removed from the tissue, which makes the use of cell lines impractical.

Organoids cannot test the impact of nematode migration through the body or replicate the changes in the extracellular matrix and cell migration that occur during injury, tissue remodelling or scarring.

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?

For Rats and Gerbils, the calculation is based on our current requirements for maintenance of parasites. The number of mice has been estimated based on experience gained under my previous Home Office licenses. This experience-based estimation has reduced the predicted animal use by over 30% compared with my previous license.

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

Maintenance of parasites: On the previous license we estimated 8000 animals for lifecycle maintenance - our procedures have improved dramatically such that we have reduced our estimate for this license to 5050. For mite feeding, we have reduced both the number of mice and the number of infected gerbils needed.

Experimental mice: For all of our experiments in-bred mice are used to reduce experimental variation, which makes it possible to use fewer numbers of 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.

We work with the NC3Rs Regional Programme Manger to ensure all lab are introduced to NC3Rs experimental design assistant and encouraged to use it. Everyone in the lab is trained in statistical methods and these are regularly discussed at lab meeting, to ensure all agree the best methods are being used. Tissue-sharing is a major tool we use to reduce animal usage.

Breeding: A significant proportion of our animal use is related to breeding programmes for GA lines. We follow the advice of our BSF staff to optimise breeding and regularly discuss numbers at lab meeting to ensure we do not overbreed. Our breeding number estimates have been considerably reduced from my

previous license. Where possible and appropriate, we use antibody blockade instead of gene knockout mice.

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

My group routinely perform pilot experiments to determine the optimal number of mice to achieve statistical power. Experiments are then performed on a minimum of two separate occasions to ensure reproducibility, following which data pooled from experiments are statistically analysed to reveal less pronounced effects without increasing overall animal use.

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.

We will use mouse models of nematode infection, fibrosis and tissue remodelling. Mice represent the most appropriate species for in vivo study of these conditions, 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.

The nematode infection models do not cause significant pathology and we use them to understand the immune response to the infection process. The infectious doses are carefully managed such that the animals will experience minimal suffering.

Our model of fibrosis will result in scar tissue and has been chosen to mimic what happens to people who undergo peritoneal dialysis. The model is terminated at a point where the causes of scarring can be assessed but before the animals experience evident suffering. Our model of severe asthma has been designed to generate remodelling of the lung airways and study the causes of airway stiffening that lead to asthma. The models are designed to allow us to quantitatively assess substantial remodelling of the lung airways, in order to mimic human disease. However, the experiments are terminated before the animals exhibit serious breathing problems.

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

We are studying long complex processes and trying to understand how the adult immune system handles these conditions and how different tissues communicate to orchestrate an appropriate response. Only adult animals would give meaningful results. The long duration of the processes to be studied prevent using mice under terminal anaesthetic.

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

In line with the establishment's policy, we will adopt the latest techniques in animal handling (eg 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 (eg for humane restraint, during and recovery from surgery). The best aseptic technique will be used during surgery.

Infection experiments will use the lowest doses possible and early endpoints will be used that prevent animals experiencing more severe harms.

We are continually refining our procedures with regard to the Litomosoides/mite lifecycle and over the years have made dramatic improvements for which we have won 3Rs awards. We are acutely aware that the gerbils are social animals and work to avoid isolation where possible. We recently moved to refine blood taking from the gerbils by switching from tail vein bleeding to saphenous vein bleeding, a procedure that is less stressful for the animal and easier to quickly get sufficient blood. Another refinement is that the gerbils are given treats after being handled and now exhibit minimal anxiety on being handled. Best practice is discussed regularly at lab meetings. In addition, the lab manager has been sent to collaborators labs to discuss and observe their practices and see if our practices can be refined. This has resulted in continual improvements to our life cycle processes.

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

The lab consults the recommended https://www.nc3rs.org.uk/3rs-resources on a regular basis including watching videos of best practice techniques. 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?

The animal facilities includes a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. My group consult closely with them and take full advantage of the extensive resources provided to ensure we are following current best practices. We are also in the process of adopting the improved rodent handling methods that reduce animal stress (detailed by Hurst et al. Nat Methods 2010) and now provide environment enrichment as standard. We will regularly consult with the NC3Rs representative and their resources page to ensure we are aware of new 3Rs developments.