Home Office

NON-TECHNICAL SUMMARY

Inflammation in parasitic worm infection and allergic disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Inflammation, Immunity, Parasitic infection, Allergy

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 overarching aim of our research is to determine which cells are involved, and which mechanisms and pathways are used, to initiate, maintain and regulate a form of inflammation, called ‘Type 2’, a distinctive immune response found mainly in parasitic worm (helminth) infections and during allergic responses. These studies will not only provide fundamental understanding of the requirements for an efficient Type 2 immune response, but should also provide proof of principle data for future therapeutic development.

A retrospective assessment of these aims will be due by 3 January 2030

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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?

There is a global need to cope with parasitic worm (helminth) infections and allergies. Helminth infections affect a third of the world’s human population, causing widespread suffering and, in some cases, lethal disease, while allergies are at epidemic levels in the developed world, and an increasing concern around the globe. This project aims to help our understanding of these conditions, as well as their relationship to one another. Given the well-established role of environmental, non-harmful microbes (such as gut bacteria) in modulating immune responses, part of this project will involve investigating whether such microbes contribute to regulation of inflammation during helminth infection or against allergens. Our ultimate goal is identification of cellular and molecular targets for rational development of novel therapeutics, which is particular urgent for helminth infections, for which there are currently very few effective treatments, as well as concerns about emerging drug resistance.

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 for helminth infection and allergies. In the 5 years of this project, 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 basic scientific questions. The new knowledge generated, and the unravelling of important fundamental immune mechanisms, will be relevant to a broad range of human and animal conditions, including helminth infection and allergies.

In the short term, the primary benefit of our work will be to discover new knowledge about fundamental mechanisms that control the initiation, maintenance and regulation of Type 2 inflammation.

In the longer term, this will ultimately provide novel candidates for the future development of innovative therapeutics targeting cells or their products (in any inflammatory disease). It also has the potential to direct drug development.

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. 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, and with local clinicians as part of the translational environment within our institution. We have highly effective systems in place for technology transfer. Additionally, we aim to expand our current collaborations with pharmaceutical and biotech companies by presentation at national and international forums at which industrial representatives are present.

Species and numbers of animals expected to be used

- Mice: 12,500 for protocol 1 (breeding) and 13,000 for experimental protocols.

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 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, including in helminth infection and in allergies. Genetically altered mice, and many of the tools designed to work with mice, allow us to define in precise detail how particular cells and molecules of the immune system work together to fight helminth infection or cause allergic responses. By manipulating these cells and molecules, we can identify the immune components that are central to Type 2 inflammation, and use that information to help design future therapies.

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 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.

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

Typically, mice will be infected with helminths (e.g. via the skin, which is the natural route of infection, or by injection) and/or be exposed to an allergen (e.g. via inhalation, or by injection), 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 (e.g. by analysing blood samples, or immune cells isolated from organs such as the lung) in the first few days after helminth 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.

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.

In most cases, only a small proportion of experimental animals will develop beyond mild symptoms. Breeding and maintenance of genetically altered mice are not expected to exhibit any harmful phenotype. Our assessment of the importance of immune cells during inflammation generated by the body in response to challenge with substances 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. Helminth infected mice may show swollen and distended abdomens. Some helminth infected animals can suddenly die from week 5 onwards, with no overt warning signs or evidence of suffering (a feature of infection that is also found in human disease). However, the majority of our experiments will not involve strains of mice, doses or times post-infection that will lead to this level of severity. In investigating lung inflammation, some animals (5-10%) may experience temporary (less than 24 hours) minor laboured breathing. The investigation of established models of intestinal inflammation ogenerally result in symptoms including weight loss, inactivity and loss of appetite, which can sometimes lead to bleeding and prolapse. Although dietary manipulation will typically be well tolerated without adverse effects, in some cases mice may exhibit marked weight gain, moderate weight loss, or failure to gain weight over the baseline. 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 Protocol.

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 severe. However, across the license, we expect the majority of mice to experience mild (approximately 40%), sub-threshold (approximately 20%), or moderate (approximately 40%) severity, with a minority of animals (<0.5%) that die suddenly, without evidence of suffering beyond moderate severity limits, being recorded as severe.

What will happen to animals used in this project?

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 3 January 2030

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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 mammalian immune system is highly complex, relying on the co-ordinated actions of multiple different cell types and molecules that collectively provide protection. Additionally, tissue environments

can play a major role in modifying immune responses. As such, the insight that animal experiments can provide on the mechanisms necessary for Type 2 immunity is of significant relevance.

Unfortunately, experiments in a laboratory dish or test tube ('*in vitro*') are unable to reflect the cellular and molecular complexity of the immune system and tissue environments in the whole body. Therefore, the use of mammals is essential for gaining a better understanding of the mechanisms underlying Type 2 immunity.

Mice will be used in these studies because their immune system closely resembles the human immune system therefore giving a better chance for translating results for future therapies. Additionally, a wide array of wild type and genetically altered strains of mice are available that will allow us to better decipher the role of immune cells and molecules in Type 2 immunity. Finally, a vast range of reagents is available for analysing murine cellular and molecular interactions during immune responses.

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

1) Use of cell lines and *in vitro* systems.

2) Analysis of human samples.

Where possible, we use *in vitro* systems to address specific questions. For example, when we identify the induction of particular proteins in a particular cell type in our animal experiments, we see if we can replicate this *in vitro* using related cell lines and the mediators observed in the animals. If we can replicate a specific aspect of our data, detailed analysis of signalling pathways is undertaken *in vitro*.

Why were they not suitable?

Our studies rely on looking at the immune response to infection or other conditions in the context of the whole body. In particular, our research aims to identify key cellular and molecular interactions processes during inflammation in tissues such as the lung, liver, intestines and female reproductive tract. Unfortunately, it is not possible to emulate these tissues and processes *in vitro*. Many location-specific features of immune cells are lost once they are removed from tissues, which makes the use of cell lines impractical. *In vitro* systems typically allow for the study of one or two cell types in a highly controlled environment that is not reflective of the complex immune system in the whole body. Therefore, to fully understand how different cell types and molecules co-ordinate an effective immune response we require animal experiments.

Experiments to track immune cell interaction, activation and function in tissues are not possible with human samples (which are often restricted to peripheral blood). Nor can we experimentally manipulate humans.

We will regularly review the literature regarding *in vitro* approaches with human and murine samples (such as precision cut tissue sections), and alternative animal approaches (such as zebrafish systems) so that, as promising new approaches are developed, we can test them and exploit them if possible.

Our research also depends on generating the egg life cycle stage of the helminths we study for use in subsequent studies. There is no alternative means of generating this stage other than in a mammalian

host (usually mice).

A retrospective assessment of replacement will be due by 3 January 2030

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 approximately 35% from our previous PPL (39,700 > 25,500).

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

For all of our 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 microorganisms (e.g. gut bacteria). 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 our Establishment 3Rs Manger to ensure all lab members are introduced to the 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. This includes animal randomisation (to reduce unintentional bias in selecting animals, that could influence results), and blinding (to reduced unintentional bias in analysis, by keeping the identity of animals/samples hidden from the researcher until the end of the experiment), 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 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.

We have many years of experience in planning animal experiments and we plan our research to ensure that all animals are used most effectively. We often combine experiments to ensure that multiple organs are used to address multiple objectives at once. Careful discussion between multiple researchers is required to avoid compromises. Due to high variability in immunological and infection models *in vivo*, especially in genetically altered models that we have yet to assess, we will adjust groups sizes as required, should subsequent power calculations indicate that this is necessary.

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. 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 (applying concepts from the ASRU GAA mouse breeding framework).

In many experiments, we will use bone marrow to carry out pilot studies *in vitro*, therefore only a few animals are used to initially test new hypotheses. Importantly, we continue to work closely with collaborators who can supply bone marrow samples, therefore reducing the number of genetically altered animals that have to bred to facilitate experiments.

A retrospective assessment of reduction will be due by 3 January 2030

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 to study Type 2 inflammation. Mice represent the most appropriate species for animal 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.

The helminth we study, *Schistosoma mansoni* in mice is the most established model for human schistosomiasis. Parasite migration, maturation, egg production, and pathological consequences of infection in the mouse are similar to the processes in humans. A proportion of *S. mansoni* infected mice may show signs associated with disease (abdominal swelling) from approximately 6 weeks post infection. Doses are carefully adjusted to minimise adverse effects.

Sensitisation and challenge of mice with allergens or allergen-loaded cells generates airway inflammation that is an accepted model for studying mechanisms underlying human asthma. Similarly, mouse models of intestinal inflammation are the foundation of innovative research into colitis and inflammatory bowel disease in humans.

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.

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

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 cells communicate to orchestrate an appropriate response in Type 2 inflammation. Although animals such as Drosophila and zebrafish do have an immune system, this is a lot less complex than the mammalian immune system and so cannot respond in the same way to the infectious and inflammatory systems that we study (e.g. helminth infection, or allergen exposure). Additionally, *S. mansoni* cannot be used to infect these less sentient animals.

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).

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

We will follow:

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/

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.

We will continue to use the NC3Rs Experimental Design Assistant to ensure we design experiments that will allow us to achieve statistical significance whilst minimising the number of animals we need to use.

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

Our animal facility includes a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. We consult closely with them 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.

A retrospective assessment of refinement will be due by 3 January 2030

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?