Home Office

NON-TECHNICAL SUMMARY

Immune regulation of health and disease in mucosal barrier tissues

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Immunology, Inflammation, Diet, Microbiota, Infectious Disease

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

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 aim of this project is to understand how the immune system in "mucosal barrier tissues", such as the intestine and lungs, acts to maintain health and prevent disease by mounting different and appropriate responses to infections, “good bacteria” that live in our guts, (commensal bacteria) and the diet.

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?

Immune responses in tissues such as the intestinal tract and lung continually act to keep us healthy. The immune system is continually being challenged by infections, as well as otherwise harmless stimulus from the diet and the "good bacteria" that reside in our guts. Disturbance of this normal immune function in humans and other animals results in a wide range of chronic illnesses.

A better understanding of how the immune system functions in health and disease has the potential to lead to new therapeutic approaches for a number of chronic diseases and is of clinical and veterinary importance.

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

- Advances in our knowledge of how the immune system functions in health and disease within mucosal barrier tissues.

- Ultimately, we hope to generate information which will inform new drug discoveries and therapies for human and animal disease.

- Communication by peer-reviewed publications, research conferences and seminars, and where possible through engagement with public

- Benefit across the scientific community in terms of advancement of research methods and generation of genetically altered animals.

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

This project aims to generate new insights into fundamental biological processes that help to maintain health and prevent disease in organs such as the intestine and lungs. As such the work has the

potential to be relevant to a broad range of both human and animal conditions.

In the **short term** the research community will benefit from this work. In the **medium term** the dissemination of knowledge will benefit the public, funding bodies and disease focussed charities and interest groups. And in the **long term**, we hope this work will allow drug developers will develop new therapies for patients.

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

Dissemination of knowledge: We aim to communicate our findings to the largest possible audience, where appropriate. Primarily this will be via the publication of peer-reviewed findings in internationally recognised, open-access journals with a broad audience. We will use open access repository sites, funder-backed open access journals and pre-print servers, where appropriate, to expedite sharing of our research and to share findings that may not fit the scope or depth of typical publisher-led journals. This will ensure even negative findings or observations generated through this project can be shared to reduce unnecessary duplication of studies.

Communication: Publication will be complemented by presentation of research findings at national and international seminars and conferences, as well as incorporation into outreach and public engagement forums such as “Pint of Science”, or engagement with patient groups. We routinely engage with social media (Twitter) and our institute’s press office to share our research as widely as possible.

Collaboration: We additionally collaborate extensively both nationally and internationally with other basic researchers, as well as clinicians and have pre-existing links with several pharmaceutical companies. Where possible we will share our findings at the earliest opportunity.

Translation: The institute has extensive infrastructure for the translation of basic research findings and for technology transfer, as well as for harnessing intellectual property and engaging with the pharmaceutical industry to expedite knowledge transfer, drug development and impact upon clinical practice.

Species and numbers of animals expected to be used

- Mice: 15000

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.

Adult mice, including genentically altered animals (GAA), are the most appropriate species for these studies as they resemble humans, as well as other animals, and biological insights have a strong track

record of translation to clinical advances.

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

In this project there are several common procedures that will be performed on mice:

i) Mice will receive molecules or drugs that activate or suppress the immune response, through injection or administration via oral routes.

ii) Mice will be subjected to infection with bacterial, fungal or parasitic organisms that model human infectious diseases, through injection or oral administration.

iii) Mice will be subjected to models of human inflammatory or allergic diseases, through administration of chemicals or allergens via injection, oral administration or administration into the airways.

iv) Mice will receive antibiotics to study the role of the non-infectious, beneficial intestinal bacteria (“the microbiota”) or receive beneficial microbes from defined cultures or transferred from other animals, to study their effects on immune health and disease.

v) Mice will receive diets with altered nutritional content, or defined nutrients, to study the effect of diet and dietary metabolites on immune cell function and tissue health.

Typical routes of intervention include administration via the oral route (ad lib in food or drinking water, gavage), intranasally or via intraperitoneal, intravenous or subcutaneous injection. Blood may be sampled via the tail vein, or fecal matter sampled passively. In some cases, animals may undergo transient anaesthesia.

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

Animals used on this project may experience adverse effects, including transient stress or pain and discomfort associated with i) sample extraction for purposes of genotyping or sample collection, ii) the experimental induction of human-relevant inflammatory and infectious diseases, or iii) associated with the delivery method, restraint or anaesthesia required for the experimental procedures. Specific expected adverse effects will vary and are outlined within individual protocols.

In many cases the stress and discomfort will be transient and range from a few minutes to up to 12 hours. However, in protocols associated with i) administration of substances to alter the immune system, ii) administration of infectious microbes, iii) experimental induction of inflammatory disease, iv) administration of antibiotics to alter intestinal bacteria and v) alterations in diet and nutrient administration, it is expected some prolonged adverse effects of mild to moderate severity will be experienced, with duration exceeding 12 hours.

A common expected adverse effect across multiple experimental protocols is weight loss, which will typically be less than 10% and resolve within 48 hours or treatment/experimental intervention, although in some protocols weight loss associated with inflammation may exceed this value. In all cases steps will be taken to refine experimental approaches to reduce weight loss as much as possible.

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)?

The studies outlined in this project will results in the following proportions of animals actually experiencing the following severity ratings:

Sub-threshold – approximately 30%

Mild – approximately 45%

Moderate – approximately 25%

What will happen to animals at the end of 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?

There are currently no good in vitro (i.e. cell lines) or in silico (i.e. computational) models that can fully and faithfully replicate the complex biological scenarios we are studying. In contrast, a mouse is a powerful and flexible model to experimentally investigate these questions using well-defined methodologies and experimental systems in a controlled environment. The mice used will also be inbred, reducing the genetic variation inherent in trying to study complex biology in human populations. Only through defining the full complexity of the immune system can we truly understand these important biological processes and make new therapeutic breakthroughs for the treatment of disease.

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

i) cell culture with in vitro derived primary cells, or cell lines.

ii) *in silico* (e.g. computational) analyses or meta-analyses of publicly available.

iii) parallel studies utilizing human materials.

Why were they not suitable?

The complex tissue environment in which immune cells are found in humans and mice cannot be fully reproduced in common non-animal approaches or accurately modelled through in silico studies. We specifically study the function of rare immune cell populations within the context of complex tissues,

e.g. the gut and lungs. While we aim to use alternative approaches – such as public data sets, cell lines, organoids, organ-on-a-chip – to expand our investigations, they do not reflect or fully capture the complexity of organs.

Furthermore, while we aim to complement our research with human samples, access to cells derived from organs such as the intestine and lung is limited and/or often not feasible in healthy humans due to the invasive nature of obtaining these samples, while tissue-associated immune functions are not accurately represented in easily obtainable human tissue, such as the blood. Moreover, humans exhibit a vast degree of genetic and life-style associated variability which can only be controlled for via the use of inbred mice within a controlled and defined environment, and it is not ethical and/or feasible to knowingly infect or induce disease in healthy human beings.

Finally, animal models also offer invaluable opportunities to genetically manipulate immune cells to better understand their functions in health and disease that are not possible, or currently technically challenging with human samples alone.

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?

Animal numbers were estimated through consultation with professional statisticians, the NACWO(s) and other named persons, and senior license holders in my department with significant experience in running a license.

The majority of animals utilized on this license will be generated through breeding of genetically altered animals (GAAs) (Protocol 1) and supplemented in subsequent protocols with wild type animals purchased from commercial vendors. As such the numbers required takes into account mice required for breeding and experimental work as well as the crossing and maintenance of new GAA lines.

Animal numbers in individual experimental protocols were calculated based upon prior knowledge of typical experimental design and experimental units associated with these procedures. Where possible, numbers were also based on typical prior and current usage of GAA lines and commercially purchased animals across each procedure and based upon typical usage over the last 5 years, where the procedures were routinely performed.

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

Experimental design was informed through consultation with an approved statistical agency. We will utilize power calculations, alongside the National Centre for Replacement, Refinement and Reduction

(NC3Rs) experimental design assistant, throughout the duration of the project to ensure each experiment is designed optimally for the central experimental readouts. In the majority of cases we will be able to utilize previously generated data from our laboratory, colleagues or publicly available data sets to inform these calculations. However, in some cases where experiments with a given procedure or combination of procedures, new GAA models etc have not previously been performed we will begin by performing a small-scale pilot experiment – often with smaller group sizes (n=3-4) if animal availability is limited - to determine whether any effect is present and to inform experimental design of future replicate or related experiments.

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

The use of GAAs, bred in-house, is critical for the success of this project. Thus, efficient breeding strategies and management of breeding colonies will be essential to minimise animal usage through the course of this project. To address this we will undertake several complimentary approaches;

i) For the generation of new GAAs via crossing of existing lines we will limit breeding to the minimum required to propagate the colony towards the final experimental genotype.

ii) To the best of our abilities we will strive to continually estimate the requirements for each individual GAA line.

iii) To reduce animal usage, experimental procedures performed for the first time without prior experimental data will be run as small scale "pilot studies" to determine the presence or absence of an effect. This pilot data will be further incorporated via sequential analysis or pooling of data when performing additional validation experiments to maximise statistical power.

In all situations we strive to share tissues from experimental animals both within the laboratory group and with colleagues and collaborators within the university. Where possible tissue samples may additionally be taken and frozen for subsequent use to maximise experimental readouts from an individual animal and reduce the need for additional animals to generate separate readouts.

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 utilize mouse models of infection and intestinal and pulmonary inflammation to study interactions between the immune system, diet and beneficial microbes that reside in the intestine. These models are the most appropriate because they have been extensively studied over several decades and their relationship to clinical human diseases and therapeutic potential have been

extensively demonstrated. Moreover, mice enable application of cutting-edge scientific tools and techniques, genetic alterations and ease of breeding and handling that make mice the most informative and ethical model for the studies proposed.

Our models of infection and inflammatory, allergic and metabolic disease will lead to weight loss or gain, gastrointestinal symptoms (e.g. diarrhoea) or short term breathing difficulties - which were chosen to mimic clinically relevant human diseases. The experimental models are designed to induce the minimum clinical signs and symptoms necessary to model human disease and the associated immune response, and all animals will be humanely euthanised prior to the onset of symptoms approaching the severity limits indicated. Throughout these studies we aim to determine how the immune system can be boosted or blocked therapeutically in order to prevent disease symptoms and suffering.

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

We are studying complex interactions between multiple biological systems (immune system, intestinal resident beneficial bacteria, diet) which can only be fully reproduced in adult mammalian species, such as mice, that have a fully developed immune system, consume solid foods - equivalent to the adult human diet, and which are colonized by commensal microbes.

The long-term nature of many of the experiments, need to study responses at multiple time points and need for sequential experimental steps prevent the use of terminally anaesthetised animals.

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

Following the establishment's established policies, we will adopt the latest techniques in animal handling and husbandry to significantly reduce the stress associated with experimental procedures as much as possible. Furthermore, where possible, the least invasive methods of dosing or sampling will be applied (e.g. repeat intraperitoneal injections will alternate sides to reduce any adverse effects), and analgesics or anaesthetic use considered to manage pain and post-operative care, e.g. humane restraint during a procedure, close management of pain, temperature and conditions where necessary).

Experiments in which animals receive an infectious or inflammatory agent will always be performed with the lowest dose possible and with end points as early as possible, to prevent the animals from experiencing unnecessary or severe harm.

While the lab has significant experience in all experimental procedures herein, we are also continually refining our procedures and will consult with the NC3Rs and institute staff to take advantage of changes in best practice or new opportunities to refine these methodologies. Best practice is discussed with lab members at regular group or one-on-one meetings and we maintain close contacts with other labs locally, nationally and internationally who run the same experimental models and discuss new advances that may help to refine experimental procedures and reduce harm.

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

The lab consults the latest literature on recommended standard practice and refinements (e.g. Morton et al Lab Anim 2011, PMID [11201285]), the recommended resources from the NC3Rs at https://www.nc3rs.org.uk/3rs-resources regularly to inform ourselves of new advances, and videos of best practice techniques, and regularly receives updates and suggestions from the institutes NTCOs.

For the specific experimental models employed here we read published research papers from laboratories worldwide utilizing the same approaches, and consult with local, national and international colleagues to discuss methods to refine these procedures.

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

We are routinely informed about advances in the 3Rs via internal newsletter emails. In addition, we will aim to stay up to date with advances via the NC3Rs website, publications of methodology and handling refinements, and attendance at seminars offered by the NC3Rs and similar organisations - both online and in person. As an example, we are 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

In addition, we will maintain regular contact with the Named Veterinary Surgeon (NVS), Named Animal Care and Welfare Officer (NACWO) and other named staff to inform ourselves of opportunities for refinement with the animal facility, and where possible discuss suggestions for refinement arising from our experimental work.