Home Office

NON-TECHNICAL SUMMARY

New Roles of the Rho GTPase Signalling Network in Health and Disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Rho GTPase signalling, Guanine-nucleotide exchange factors, Bacterial infection, Inflammation, Glucose metabolism

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

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?

Our aim is to increase our understanding of the molecular and cellular processes that enable a healthy lifespan and that, when deregulated, can cause or worsen diseases such as immuno-deficiencies, chronic inflammation and type-2 diabetes.

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?

A better understanding of the biological mechanisms underlying human health and the causes of complex diseases will allow us to better treat such diseases in the future, by enabling us to use rational strategies in the development of more efficient therapeutics with fewer undesirable side-effects.

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

The expected benefit from this project is that we will gain a better understanding of the complex molecular and cellular mechanisms that underpin a healthy lifespan and that - when deregulated - can cause or exacerbate complex diseases such as immuno-deficiencies, inflammatory and metabolic disorders. Our findings will be published in open-access journals. In the long term, the knowledge generated from this project might contribute to the development of more effective and less toxic drugs.

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

The immediate beneficiaries are the scientific community and the interested general public who will gain new understanding from our open access publications, presentations and conferences and public engagement activities. In the longer term, the pharmacological industry may benefit by deciding to develop new therapies on the basis of our findings. Eventually, society as a whole may benefit from the improved health brought by such therapies.

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

We will publish our findings in open-access journals accessible to all and disseminate our results at international scientific conferences. We will collaborate extensively with other laboratories around the world to maximise the impact of our work. We will make our research accessible to the general public, with the help of our public engagement team.

Species and numbers of animals expected to be used

- Mice: 3630 per year

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 will use mice, as they are the best understood and most widely used mammalian laboratory animal, with excellent means for generating and interpreting the effects of genetic modifications. Most of the mice will be used at young adult life stage, some will be compared to old mice, and a few will be tested throughout their lifespan. We will compare young and old mice because some phenotypes, such as inflammation and metabolic syndrome are age-dependent.

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

The majority of mice will be used for the generation and maintenance of genetically modified strains or for the collection of cells and tissues for analysis ex vivo after the animals are humanely killed. Some mice will be aged, so their inflammatory, immune and metabolic responses can be compared to those of young mice. Some mice will be given single injections to challenge their immune system for observation during a few hours or days, or for altering their blood sugar levels. Some mice will be tested throughout their lifetime by blood sampling, either on a healthy or unhealthy diet, in order to monitor the effects of ageing and diet on their blood sugar levels. A few mice may be used for imaging under anaesthesia, for close monitoring of their inflammatory or immune response.

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

The main adverse effect could be a mild and transient inflammatory response. Few animals may experience a more sustained inflammatory response. Rarely, animals may experience health problems associated with weight gain during ageing caused by access to unlimited food supply. All animals will be killed by a quick and humane method at the end of experiments.

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)?

Around 60% of the genetically modified mice will experience mild severity at worst from being bred and maintained in our excellent animal facility and are not expected to show any overt phenotypic signs due to their genetic modification. Around 20% of mice will experience mild severity at worst from a mild and transient inflammatory response. Approximately 10% of the mice will experience moderate severity at worst from a more sustained inflammatory response, and round 10% are expected to experience moderate severity at worst associated with weight gain during ageing caused by access to unlimited food supply.

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?

Physiological context is important in our research, because we study biological processes that affect the whole body, namely inflammation, the clearance of infections and metabolic homeostasis. The complexity of these processes cannot be adequately modelled by other means such as tissue culture, purified proteins or computational methods. Thus, we need to use animals, and in order for our data to be relevant to human biology, we need to use mammals rather than non-mammalian species in which these processes are very different. The mouse is the mammalian species most widely used, most amenable to genetic modification and best understood for such research. Thus, we must use isolated primary cells isolated from mice and research involving in vivo experimentation in mice to achieve the aim of our project.

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

We use cell lines and purified proteins widely and wherever possible to study the Rho GTPase signalling network.

Why were they not suitable?

Tissue culture and purified proteins are very valuable in some aspects of our research. However, there are limits, as the cell types that we are most interested in are terminally differentiated and cannot be cultured, and their protein composition cannot be modified other than by genetic means. Our main cell model, neutrophils, are also very short-lived. Hence, we must resort to primary cells that we isolate freshly from genetically altered mice and compare these with cells from wild type control mice. In addition, isolated cells, whether primary or cultured, cannot give the physiological or pathophysiological context of the complex biological systems we are interested in. To understand this context, in vivo experimentation is the only option.

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?

We will use group sizes that can confidently detect significant differences, determined largely by past experience, and also from the published literature, with help from the Institute's statistician where needed.

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

We strive for minimal variations in age, sex and genetic background between groups to reduce variability. We use blinding and randomisation of samples where possible to minimise bias. All scientific staff are trained in statistical methods and have support from our statistician to design experiments and analyse results. We cryopreserve mouse strains that are not in current use, in order to reduce numbers of actively breeding strains.

During our previous project licence, we made two particular advances to reduce animal numbers:

The proteins we work on regulate a wide range of physiological and pathophysiological responses. Our research has identified a novel regulator of one of these proteins. We had planned to study the role of this protein in neurons using genetically altered mice. Instead, we generated a genetically altered neuronal cultured cell line by CRISPR knockout, which allowed us to study the role of this protein i*n vitro*, without the need for primary cells. This has eliminated the need for generating and testing the genetically altered mouse strains.

In collaboration with our statistician, we have reduced the number of control animals needed for certain *in vivo* experiments. This became feasible when new versions of GraphPad software were developed for such purposes, which allow meaningful statistical analysis when the size of the control group is smaller than the test group. For example, in experiments where we know the response to be minimal in the sham-treated groups but high in treated groups, we now routinely use a single mouse in the sham-treated group per experiment.

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

We perform pilot studies for models that are new to us, to determine numbers of mice needed and to optimise conditions. Increased use of *in vivo* imaging will allow us to obtain richer data sets from each mouse and thus reduce the number of mice used. We will share tissues with other laboratories wherever feasible. We cryopreserve mouse strains that are not in active 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.

Wherever possible, mice will be used for isolation of primary cells and tissues after they were humanely killed, rather than for *in vivo* experimentation. The minimum number of animals will always be used that yield meaningful results, and with the lowest possible relevant severity procedure to address a specific question.

We will work in cell lines to evaluate potential functional impacts of genetic modifications prior to generating new mouse strains. In new genetically modified mouse strains, we will undertake pilot studies and test isolated primary cells to evaluate phenotype prior to using relevant *in vivo* models, starting with mild severity procedures and progressing to moderate severity depending on evidence gathered.

We use bacterial infections to challenge the immune system of mice and evaluate their ability to clear these infections. These infections would cause some of the mice to develop clinical signs and even die, if these mice were kept for several days after the infection. We will limit the period of time that infected animals are kept so that we can detect an inflammatory or immune response, without clinical signs or deaths expected.

Bone marrow transplantation may cause up to 5% of mice to die from failed engraftment. Use of this procedure is justified because it informs on the cell types involved in an inflammatory or immune response, thus minimising the need for additional mouse strains. Treatment of the mice with antibiotics and careful timing and dosing of the transplant material will be used to refine this procedure and minimise harm.

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

The mouse is the best model organism to address our aim and objectives, as its physiology and disease processes are sufficiently similar to humans to allow us to draw meaningful conclusions, and because a wide knowledge base and many genetically modified strains and protocols exist that allow comparisons of results between projects and research groups.

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

Our routine refinement methods include added enrichment for breeders, alternative bedding for animals with reduced motility, using established social groups where possible, habituation to handling, and providing food in gel format, additional warmth and more frequent monitoring for mice at increased risk. For established strains, we only biopsy mice from the first litter for genotyping, and we use ear rather than tail biopsies. We may introduce further refinement methods to protocols or husbandry methods in consultation with animal technicians and veterinary staff.

Stress and suffering of mice undergoing procedures will be minimised by observation and adherence to clear guidelines on clinical signs that trigger the end of an experiment. Mice whose immune system we challenge will be monitored closely for body weight or adverse behaviours. Mice for long term evaluation of blood sugar levels will be habituated to handling, and we use a refined method of blood

sampling to reduce stress to these animals. Mice on these long-term studies, and all our breeders, have extra enrichment in their cages to further eliminate stress. In rare cases where it will be required that we induce and maintain general anaesthesia, we will use modern anaesthetics and continuous monitoring.

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

We will follow PREPARE and local campus guidelines.

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

I periodically check the NC3Rs website which gives excellent advice and advertises upcoming seminars, helping me scan for advances and new ways to implement the 3Rs. In addition, our Home Office and AWERB liaisons keep us informed of 3Rs seminars and events.