Home Office

NON-TECHNICAL SUMMARY

Understanding Infection Pathogenesis to Guide Therapies

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

Infection, Immunity, Pathogenesis, Therapy, Vaccine

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

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 overall aim of this project is to understand and define the mechanisms involved in the host / pathogen interaction and to explore novel therapeutic approaches.

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?

Infection remains a major cause of death worldwide. The latest available data from the World Health Organisation (WHO) shows that in 2019 infections accounted for 3 of the top 10 leading causes of death worldwide with a total of 6.1 million deaths annually (https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death). As of 2019, infections were accountable for 6 of the top 10 causes of death in low-income countries. Even in the UK, lower respiratory infections are still in the top 5 leading causes of deaths each year.

Over the last 10 years, we have started to see a sharp rise in pathogens that are resistant to antimicrobials. These pathogens are not just resistant to one or two antimicrobials, but we are seeing the emergence of pathogens that are resistant to multiple antimicrobials with resistance in some pathogens, such as TB, to even the last few remaining first-line antimicrobials currently in use. This is confounded by the fact that there have been very few new antimicrobial products that have been developed over the last 15 years. A review by the UK government on emergence and economic cost of antimicrobial resistance can be found here (http://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf).

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

We believe this project is in-line with the United Kingdom/global aim to use genomics to improve medical treatment and human health, and to develop therapies that will benefit the economy and human health.

We strongly believe that the work described in this proposal will both improve our understanding of infection related illness and help identify novel targets for new therapeutic approaches. We expect to characterise up to ten novel genes that are also in humans which influence infection, immunity and pathology. We will propose ways which these genes can be targeted for therapy when we have further defined their functions and, through publications, will make these findings available to the wider scientific community. We will identify pathogen genes or gene products that contribute to disease, and will define the way by which they escape therapy (resistance). We have a track record of using pathogen products in vaccine trials and in the formation of companies with the potential to take products to clinical trial.

We will share the data we generate with other researchers around the world through open access research databases and publications. Some of the bacteria we will be working on have no current vaccines and data we will generate here may lead to clinical trial and usable vaccines for public health.

All mouse lines used will be shared with other research groups.

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

We believe that the work described in this proposal will both improve our understanding of infection related illness and help identify new targets for new therapeutic approaches such as vaccines. In the short term we will publish data on novel genes associated with pathogen infection. We will identify novel pathogen proteins that either improve on current less effective vaccines or develop vaccine products for pathogens where no current vaccine is available. In the longer term we believe that products developed under this licence, be that either vaccine or immune modulators, will be used to prevent disease or enhance immune responses in humans. We will share the data we generate with other researchers around the world through scientific publications or with companies with the ability to produce therapeutics.

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

This work will be part of a larger collaborative program including programs working on cells, fish infection models and human trials. The results will be shared at the earliest opportunity through publication and at conferences. Large data sets will be deposited on databases for external access. We will disseminate all findings of our studies, including unsuccessful approaches or non-significant data, through publication in peer-reviewed journals, presentation at scientific conferences, and through meetings with other researchers.

Species and numbers of animals expected to be used

- Mice: 10680

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.

To better understand our body’s ability to fight infection or to mount immunity, we cannot simply start experimenting on humans, but need to use a model system. The more similar the model is to humans, the more reliably we can make conclusions about humans based on the findings of our study. We have selected the adult mouse as a model animal for our work as it has a fully developed immune (bug defence) system similar to that of humans. In fact, the mouse immune system is made up of the same parts as the human one and uses the same strategies to get rid of bugs, bacteria and viruses in our body. The mouse is also widely used and very well characterised, which means many different

scientific tests have been developed for it which we could make use of to investigate in our study. For example, the well characterised genetic code of the mouse allows us to look into the role certain genes and inheritance plays when it comes to vulnerability to infection.

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

Animals will be produced by traditional breeding methods. We will make a mouse mutant in a gene that we have identified from human immune studies that we think is important for controlling infection. This will allow us to study the role of that gene in a controlled way.

Animals in this project will typically be infected with a bug (virus / bacterium) or vaccinated. This will involve either an injection, for example intravenously (i.e into a vein), or administration intranasally (i.e into the nose) or by oral gavage (a tube inserted via the mouth into the stomach). Vaccinations will be given via injections into the muscle or under the skin. Samples of blood will be taken from a tail vein (blood vessel) at predetermined time points. Animals will be monitored during the infection period by weight and in some infections by sampling of faeces. We can also track some bugs over time by imaging the mice with imaging machines while the mouse in under anaesthetic. To better understand the role of some genes in the immune system we may need to irradiate mice (a way of killing only the immune cells of the mouse) to replace their immune system which means we can track cells to understand their role in infections.

At the end of the experiments all mice will either be humanely killed, or blood collected under deep, terminal anaesthetic where they will be asleep/unconsciousness throughout. The experiments will be typically 1 month long.

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

As the mice will be infected with bugs they are likely to suffer some discomfort. In wild type mice all the infections we use cause disease but do not kill the mice. Mice infected with bacteria will have moderate weight loss of 5-15% over 7-14 days and will have mild clinical signs of infection; slight piloerection (hair standing on end as a sign of pain or discomfort in mice), and hunched walking. With flu infections the weight loss will be 15-25% less than the starting weight over 10 days and mice may develop increased breathing rates.

In gene deleted mice where the gene is important in controlling infection, we may expect to see increased weight loss and more pronounced clinical signs. We will have pre-defined end points for our infections to capture animals and minimise any suffering.

Mice that are immunised can show mild signs of discomfort that should last no more than 24 hours. These mice are not expected to show any long term adverse effects.

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)?

Mice

Mild 70%

Moderate 30%

Severe 0%

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

- 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?

We only use live mice when we cannot use alternative approaches such as working with human or animal cells in a dish (these could be blood cells or cancer cells for example that have been taken from patients). It is not possible to mimic all aspects of the interactions between the different stages of a real infection process meaningfully outside of the whole animal. Additionally, it is not possible to study the complex interaction between bugs and the host that they infect (e.g. humans) outside of a whole and living animal. We recognise that our mouse model system has limitations and cannot reproduce all the conditions associated with parallel human infections or vaccinations. However, mice have similar immune systems to humans making observations in mice comparable to those in humans. As mice don’t always react the same way to bugs that attack humans, we use mouse-specific pathogens to evoke a more meaningful response that can be paralleled in humans. These mouse-specific pathogens include an adapted version of the flu virus that causes similar symptoms in mice to the ones humans experience from the flu, or an adapted bacterium that mimics stomach infections causing diarrhoea etc in humans.

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

Cells and organoid systems (recreating organs or parts of them from cells in the laboratory, for example part of a gut) have been considered.

Why were they not suitable?

It is not possible to mimic all aspects of the interactions between the different stages of a real infection process meaningfully outside of the whole animal.

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?

By keeping our experimental conditions well controlled we are able to perform highly reproducible and statistically meaningful experiments using the minimal number of animals. The experimental approaches described herein have been vigorously evaluated over the past two decades. We have access to and have used an experienced statistician to help guide experimental design. Post-doctoral scientists in our group have been on experimental design courses to help understand sources of bias and variation and how best to reduce them. Where possible in our design we blind people to genotype/treatment groups with different people doing infections or analysis. Based on our experience with pathogen infected mice, we use 5-6 mice per group as this is a sufficient sample size given the differences between means and within-group variances we typically observe. We will share any new mouse models we generate with other researchers.

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

We have been running these infection models for the last 20 years and during that time we have generated thousands of data points in wild type mice in normal conditions. From this we know what the sources of variations are and have therefore been able to control for them. We have worked closely with Biostatisticians when setting up high throughput screens using these models to reduce the numbers of mice we need to use to get meaningful results. We have used the NC3R’s experimental design assistant for work we have done on previous studies and will continue to use it in future studies. The PREPARE guidelines have also been consulted for formulation of this project, and these will be followed to ensure continued communication between the animal facility and our team.

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

The work is also a part of a larger program of work within the department where data will be generated for other experimental models including work in fish and humans along with experimental models in vitro such as cell and organoid culture. The data generated in these programs will also inform the work herein. When measuring something new or it is unclear what the correct dose will be to achieve our aim we will use a small pilot study to help guild decisions. Samples collected from any mice as part of experiments planned will be stored long term at -70oC. This will make the samples available for future analysis. There is potential that other groups may want to use post-mortem tissues that we do not take from mice that have been exposed to infections.

We are engaged with the local NC3Rs group who enable users to share surplus breeding animals to reduce numbers.

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 have established that the mouse can be used to identify bug (also referred to as ‘pathogen’) and mammalian genes that influence infection and immunity. The wild type parental bugs that we use are almost exclusively disease-causing for the mouse and thus likely to yield important and relevant phenotypes and associated data. Although the pathogens we use are mainly mouse pathogens they are good correlates to human specific diseases. We believe that the similarities in the mouse and human genomes are such that we can infer between the two and we have closer links than ever before with patients in the clinic. Over the years we have gained tremendous experience with our infection models and, through careful observation, we are able to minimise the potential suffering of the animals. We have been able to identify key clinical signs that indicate illness in infected animals and consequently such animals can be quickly and humanely killed.

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

It is not possible to mimic all aspects of the interactions between the different stages of a real infection process meaningfully outside of the whole animal. It is not possible to study the complex interactions between the host (e.g. humans or mice) and infectious agents / bugs (e.g. the flu virus) outside of whole living animals or in animals that don’t have a mammalian immune system. We recognise that our mouse model systems have limitations and cannot reproduce all the conditions associated with parallel human infections or vaccinations. However, mice have many similarities to humans, including in terms of their immune system. We also use mouse-specific bugs to evoke a more meaningful response that can be paralleled in humans.

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

Mice are monitored throughout all experiments and we collect daily scores composed of a set of physical signs of illness such as piloerection (raised hair as a sign of pain or discomfort), hunched walk and mobility along with weight loss. The cut-off for these physical signs lies within the guidelines for moderate severity, i.e. loss of pre-set percentage body weight being our main indicator, along with mobility (ability to feed and water). The scoring for piloerection etc. are also used as secondary indicators. My team are experienced in animal infection models and are trained to the high standards that I expect. The technicians that work in our holding facility and do the majority of the animal husbandry will also be trained by my team and will communicate abnormal behaviours in the mice early. At our establishment we have dedicated Named Animal Care and Welfare Officer who are

impartial and can give advice/ make decisions on animals that lie outside of the normal adverse effects expected for the infections outlined within this project.

Potential refinements include increased monitoring if test animals show earlier clinical signs or weight loss. We will give wet mash food to animals that lose more weight quickly. Floor food will be given to animals that are to be infected to limit weight loss from the start of the infection or the use of medicated palatable substances for voluntary treatment such as flavoured jelly, paste or milk shake liquid where the use would not impact on the scientific aims.

All animals acclimatised to the facility before use and will be given environmental enrichment and be socially housed to encourage natural behaviours and reduce stress. Males will be monitored for increased aggression and additional enrichment added to combat or appropriate splitting of fighting animals if needed.

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

We will use guidance from the NC3Rs website and the Laboratory Animal Science Association (LASA) to ensure experiments are conducted appropriately. In particular we will follow the 'Guiding principles on good practice for Animal Welfare and Ethical Review Bodies'.

We will follow the PREPARE guidelines for planning experiments and will follow the ARRIVE guidelines reporting of results.

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

I will keep informed on advances through the NC3R’s website, Norecopa website, our establishment website and newsletter. We will discuss any advances with the relevant people at our establishment and implement them accordingly.