Home Office

NON-TECHNICAL SUMMARY

Host and Bacterial interactions in mycobacterial infections

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.
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)

Key words

tuberculosis, macrophage, granuloma

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 project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

TB continues to be a major disease throughout the world and more information is required as to how the causative agent, *M. tuberculosis*, develops during infection. In order to address this, we will examine the relevance of mouse and mycobacterial genetics/ proteins on bacterial growth. Genes will be deleted from mycobacteria to assess the impact on growth and also the effect of the absence/presence of mouse proteins/genes will be examined.

A retrospective assessment of these aims will be due by 06 January 2025

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's 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.

What are the potential benefits that will derive from this project?

M. tuberculosis continues to infect and kill millions of people each year. This is the leading cause of deaths in HIV co-infected individuals and is further complicated by the occurrence of multi- drug resistant and extensively drug resistant strains. There is an urgent need for a new vaccine and new anti-tuberculosis drugs. Animal model studies of tuberculosis can provide substantial advances in these areas. This project aims to further knowledge regarding the effect of mouse (host) and mycobacterial genes/proteins in development of infection. The long term aim is for this knowledge to be used for developing new strategies to combat TB.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Mice, 5000/year

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The project will use a mouse aerosol TB infection model to examine the effect of deleting mycobacterial genes involved in bacterial metabolism and/or host (mouse) genes on growth of mycobacteria and development of disease. Genes considered important for mycobacterial growth will be deleted and those found to grow less in culture, when compared to the original strain, will be examined in the mouse model using wild type mice. Other studies will use mice which have had specific genes deleted or inserted to compare these to the control mouse strain. The genes altered will be related to proteins considered important in cells that regulate the response to mycobacteria. These mice do not have any abnormalities due to these changes. In addition, well characterised compounds that can alter the host response to infection will be tested in the mouse model. The gene changes and modulators are most likely to be beneficial to the mouse as these are expected to decrease the amounts of bacteria during infection. Mice exposed to a low dose aerosol infection can survive for more than 1 year without showing signs of discomfort. Some of our studies may run for approximately 6 months.
The majority of mice in these studies will be wild type mice, relatively resistant to TB infection, undergoing a standard aerosol infection that results in no change in condition of the mice over the duration of the study. Some studies using this model will require mice to be administered modulators. These modulators are not expected to cause increased severity; indeed these may decrease the amounts of bacteria in treated mice. Multiple vaccinations may be required as some compounds may only be active for short periods of time. Throughout the studies mice will be monitored for any signs of physical or behavioural changes and weighed to ensure they are not becoming severely affected by the procedures. Where more sensitive strains of mice are used these will undergo shorter infection programs and routine monitoring to ensure they are not severely affected. There are some instances where we may need to assess more virulent strains of mycobacteria and under these circumstances mice used in these studies may experience more severe symptoms. As soon as there is an indication of this severity, from their physical and behavioural readouts, these mice will be humanely killed. All animals will be humanely killed at the end of a specific set of procedures. Animals exhibiting any unexpected harmful abnormal phenotypes will be killed and advice will be promptly sought from the NVS and local Home Office Inspector.

A retrospective assessment of these predicted harms will be due by 06 January 2025

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 why you need to use animals and why you cannot use non-animal alternatives.

Most of the exploratory work will be performed in vitro with isolated cells from either mice or human samples (e.g. blood). We will generally be examining compounds that already have been tested by others but not necessarily in TB studies. Initially, the substances of interest that we believe could result in an improved response against TB will be examined using cell culture systems. Information from

these studies will guide our mouse infection studies. However, the response by the body to TB infection is very complex and requires various cell types to come together and function at different times during the infection. So ultimately to be able to perform detailed immunology and pathology investigations we need to use animals e.g. the effectiveness of vaccines and antimicrobials cannot be carried out without the use of animals. Also, although compounds that eventually will be tested in the mouse model will already have been screened for suitability using cell culture tests. These compounds may be less effective in mice than in cell culture so we cannot replace the animal tests; where the real conditions of an infection are present.

To achieve the objectives of this project, we propose to use the laboratory mouse as the model organism. This model is the best-characterised model for TB studies with many components of the infection being similar to that of the human infection. Aspects of the mouse model that make this accessible are: techniques for producing genetically modified mice are well established; mice have a relatively short generation time and their biology has been extensively studied. There is also a range of reagents available for mice specifically that make studies in this model much more accessible than other species. To our knowledge, no other species of lesser sentience can fulfil the requirements of this project to the same extent as the mouse.

A retrospective assessment of replacement will be due by 06 January 2025

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 you will assure the use of minimum numbers of animals.

Only samples that have undergone prior screens in culture to meet the criteria for progressing to testing in the mouse are used. Colonies are managed to produce enough mice for each study with minimal excess. The numbers of mice used in our studies are based on the PREPARE guidelines where the sample size is defined by performing a power calculation. This calculates the number of mice required to give a significant result - where the result is unlikely to be due to chance. Based on this calculation and the results of the extensive data already generated from our studies we require at least 5 mice per group for each study point. Where possible to reduce numbers, we use the same sample for multiple types of tests and samples may be stored for reassessment or made available for other studies if applicable. Also using new imaging technology to study each infected mouse will ultimately decrease the numbers required for studies.

A retrospective assessment of reduction will be due by by 06 January 2025

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

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Most of the exploratory work will be performed in vitro with isolated cells from either mice or human samples. Mice are the least sentient species available for studying TB with the best set of reagents available for analysing their response to TB. We have chosen the mouse aerosol infection model to study TB disease as this model is the best characterised model for TB infection studies with many features shared with human infection. The mouse immune system is well defined and the technology available for assessing this is highly developed. Additionally, the use of aerosol exposure, to produce an infection, makes this a physiologically relevant model. This route is also less invasive than others in use e.g. intratracheal. We respect maximum volumes indicated for each route of administration and the experiments are only performed by highly trained professionals. The low dose aerosol is well characterised so we are aware of how mice should respond. In these studies, the growth of the organism in the mouse model can lead to disease so body weight, level of activity and general appearance will be closely monitored to minimise harm. If any alterations are made to the protocol then this is refined to ensure changes do not lead to increased suffering. Initial infection studies can guide us to reduce timescales and numbers for future studies. Certainly, the use of new imaging technology will help us gain more information as to how the disease progresses and will ultimately aid in modifying the system and decreasing the numbers required for studies.

A retrospective assessment of refinement will be due by by 06 January 2025

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?