Home Office

NON-TECHNICAL SUMMARY

# Maternal and neonatal immunity to virus 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

**Key words**

virus, antibody, infant, vaccine, drug

| 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 aim of this project is to characterise maternal and infant immune responses to virus infection in order to develop novel approaches for control of virus infection in both mothers and offspring.

**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?**

Childhood virus infections are a major cause of infant mortality. For example, worldwide an estimated 215,000 children under 5 years old die annually from diarrhoea caused by rotavirus infection (Tate et al, 2015), and up to 111,500 children under 5 die from respiratory disease caused by influenza infection (Nair et al, 2011). In addition, the extent of disease in children caused by newly emerging viruses such as COVID-19 is not yet fully known.

In order to protect children from virus infections it is essential we develop highly effective vaccines and anti-viral drugs. We therefore need to understand how the immune system of infants responds to virus infection so that the most effective control strategies can be designed. It is known that the maternal immune response can significantly affect the immune response of offspring, so both groups of individuals must be carefully evaluated together. Using a breeding mouse model of virus infection will enable detailed study of this complicated system.

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

By the end of this project we will have greater understanding of the immune responses of mother and infants to virus infections. We will use this knowledge to develop a new approach to protect babies from one or more of the viral infections of childhood. This may be in the form of a new vaccine strategy, or a new anti-viral drug. These findings will be published in peer-reviewed journals following the ARRIVE 2.0 guidelines (a checklist of recommendations to improve the reporting of research involving animals), and we plan to put forward any promising new products for clinical trials.

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

Improved knowledge of mother and infant immune responses to viruses will be of considerable value to the wider viral immunology research community within the time-frame of this five year project. Development of new vaccines or anti-viral therapies has the potential to reduce childhood disease and deaths from virus infections, although delivery to patients will require lengthy clinical trials which will occur beyond the duration of this project.

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

Partnerships are already established with a collaborator who plans to study new vaccine approaches to virus infection in adult mice. This project will therefore complement ongoing work.

Knowledge gleaned from this project will be published open access in a timely manner in order to disseminate findings as widely as possible. Both successful and unsuccessful approaches will be published and discussed at scientific conferences.

**Species and numbers of animals expected to be used**

- Mice: 8880

# 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 study antibodies that are transferred from mother to infant across the placenta and in breast milk ('maternal antibodies'), a mammalian model is required. For this reason, we have chosen to use the mouse as this is a good and widely accepted mammalian model for this research. This model will enable us to gain understanding of virus infections and maternal antibodies in humans.

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

Female mice will be infected with different viruses to induce protective immune responses. Mice will then be mated in order to study how maternal immunity is transferred to their offspring. Immune responses in both mothers and pups will be assessed by collecting small volumes of blood from the animals during life, as well as extensive study of their organs after the animals are killed at the end of each experiment.

Pups may be infected with virus within the first week of life, or later as adults. Virus infection levels will be measured either by studying stool/blood samples, imaging mice under anaesthesia, or from organ analysis after death.

New vaccine approaches will be tested by either vaccinating the mother before/during pregnancy, or by vaccinating young pups. To test how effective vaccines are, pups will be infected with the viruses when adults.

New anti-viral drugs will also be tested in mouse mothers and pups to see if new ways of treating virus infections can be identified.

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

Some of the viruses studied may cause mice to lose weight in the first two days after infection, but weight normally returns back to normal within approximately 7 days. Some of the viruses used may also cause signs such as ruffled fur and rapid breathing during the same period. All mice that show these signs will either be killed if signs reach predetermined humane endpoints such as weight loss of 15% plus clinical signs of disease, or if the mice have not returned to full weight within 2 weeks.

Although mice infected with some viruses may develop signs of disease at high doses of virus, this project aims to identify improved vaccination and treatment options. It is therefore anticipated that only the control mice which don't receive the vaccine or treatment in each experiment are at risk of experiencing disease. Furthermore, careful calculation of virus doses will be performed in preliminary experiments to ensure that disease is kept to a minimum.

**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 severity 90%

Mice: moderate severity 10%

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

- Killed
- Used in other projects
- Kept alive

# 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 use of animals is essential to study the overall immune responses to virus infections. This is because the immune response to viruses is very complex and cannot be replicated in cells in a laboratory. Furthermore, to determine how effective new vaccine approaches are, experimental infections in mice are required.

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

We have considered, and will initially use cells in the laboratory for all preliminary experiments. This is because different cells can readily be infected with the viruses we are studying, and this will allow us to

study the effects of viruses and antibodies on a single cell level.

**Why were they not suitable?**

Experiments in the laboratory will be suitable for first testing whether virus replication can be blocked by specific antibodies or drugs. However, to study complex immune responses that involve many different types of cell working together, infections and vaccinations of live animals are essential.

# 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 have extensive previous experience of infecting mice with viruses, which enables us to make good estimates of how many animals will be required for each experiment. In addition, we have used a specialised programme called the NC3Rs Experimental Design Assistant to help us calculate the number of mice needed.

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

Extensive review of the scientific literature has been conducted to enable predictions for likely outcomes of experiments planned (e.g. suitable virus doses, efficacy of maternal antibody transfer), so the minimum number of mice can be used.  I have also sought the advice of more experienced colleagues to ensure animal numbers are at a minimum and welfare is maximised.

We will test all new medicines for treating virus infection in cells in the laboratory first, and only progress to animal experiments if positive results are obtained.

Where possible, we will infect mice with viruses that can be tracked using specialised imaging equipment. This will allow us to monitor how much virus is present in each animal by imaging, instead of having to kill the mice to measure the amount of virus in each organ.

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.

We have used the NC3Rs Experimental Design Assistant to help us calculate the number of mice needed, and will continue to use this throughout the project.

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

Breeding programmes of genetically altered mice will be optimised to ensure as little over-breeding as possible. This project will aim to keep “surplus” animals to a minimum. In order to reduce the numbers of breeding pairs the mice will be kept as purebred lines (when appropriate), provided that they do not show any signs of disease.

Samples collected from any mice as part of experiments planned will be stored long term at -20C. This will make the samples available for future analysis by scientists working on this project, and also for any collaborators.

# 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.**

This project uses mice that will be infected with different viruses, then their immune responses will be studied by collection of blood samples, and analysis of different organs after death.

The mouse has been selected for this project as it is the best way to study human anti-viral immune responses. The mouse also benefits from well-established and robust technologies for modifying their genes. Where possible we will use experiments of the shortest duration so long as to do so will yield satisfactory data.

In order to understand how maternal immunity impacts on infant immunity to virus infections, we must work with viruses that can replicate in mice. These can cause clinical signs at high doses, but is essential that we follow the outcome of infection to fully investigate immune responses that can lead to viral clearance. If we kill animals at the first sign of infection we cannot investigate what responses are required that allow them to clear the infection naturally. Previous experience has shown that weight loss can correlate closely with the amount of virus in a mouse. Monitoring weight loss is therefore an

effective way of determining infection, and is a widely used and accepted measure. However, weight loss is not the only measure of infection and we will use a comprehensive monitoring and scoring system to assess the animals throughout experiments.

Finally, this project aims to use non-surgical embryo transfer as a new approach for breeding genetically modified mice. This method will be explored and optimised over the course of this licence as a refinement to the existing surgical method of embryo transfer.

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

Study of how maternal immune responses impact on the immunity of infants requires use of animals that transfer antibodies to their offspring via the placenta or in milk. This specialised method of antibody transfer therefore means species such as fish, flies or nematodes cannot be used for this project.

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

All mice that are infected with viruses will be monitored twice daily for any clinical signs of illness (e.g. ruffled fur and weight loss in adult mice, or rejection by the mother in pups). The results of these will be recorded in a spreadsheet for each experiment. Additional mash will be provided for sick animals unable to access the food hopper, and extra nesting material will be given to improve the comfort and warmth of virally infected mice.

Weights for all animals over 3 weeks of age will be recorded once daily after infection until weights have returned to normal. Mice will thereafter be weighed once weekly. Again, a record of weights will be kept.

To assess levels of virus we will use minimally invasive sampling, such as sampling from faeces, or imaging where possible.

For any procedures requiring anaesthesia, mice will be monitored closely for the duration of their recovery.

For viruses given by mouth, a drop of sugar solution will be placed on the end of the delivery tube in order to encourage swallowing.

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

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

To ensure refined experimental design we will follow the PREPARE guidelines for planning experiments, and for thorough, responsible reporting of results we will follow the ARRIVE guidelines.

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

We will continue to be informed about the 3Rs during the duration of this project by regularly checking our institute's 3R's search page, and being registered for the regular NC3Rs e-mails and newsletter updates. Regular reference to guidance documents provided by Laboratory Animal Science Association (LASA) and the RSPCA will be made.

We will also ensure continued contact with the organisational teams in the facilities in which our mouse work is conducted. Any new recommendations will be incorporated into our experimental plan wherever possible.