Home Office

NON-TECHNICAL SUMMARY

Immune responses to infection and during inflammation

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 following aims mentioned in paragraph (b)

Key words

Virology, Immunology, Vector biology, Inflammation, Dermatology

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

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.

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?

This project will study mammalian host immune responses in the skin to define new therapeutic targets. We have a particular interest in host responses following infection with virus, focusing on responses to arthropod-borne virus and arthropod-derived factors. Comparisons to chronic inflammatory diseases that involve similar biological processes will also be made.

A retrospective assessment of these aims will be due by 02 August 2026

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.

Why is it important to undertake this work?

Virus spread by arthropods, called arboviruses, are a large group of emerging, medically important pathogens for both humans and economically important livestock. There exist few medicines or vaccines to treat and/or prevent infection with arboviruses. There is a clear unmet need to better understand how these viruses cause disease, and new studies that identify new medicines is urgently required. This project will define key aspects of infection that will enable us to design new medicines. In addition, the underlying biological responses that underlie antiviral immune responses are also dysfunctional in other skin diseases such as psoriasis and systemic sclerosis. Therefore this work will aid our understanding of immune responses to both acute virus infection and chronic inflammatory diseases.

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

By studying the fundamental mechanisms underpinning these processes we can better understand the complex interplay between mammals, viruses and their mosquito vectors and in doing so will help us to;

(1) define new mechanisms of innate immune function during the early events of an important group of emerging infectious disease

(2) identify the most relevant aspects for disease control and prevention

(3) identify mosquito-derived factors in their saliva that modulate mammalian host susceptibility to virus infection

(4) identify putative biomarkers to aid diagnosis and risk stratification of infected patients

(5) identify commonalities in skin responses to those that cause inflammatory skin diseases, such as psoriasis

(6) provide a highly relevant model system for studying innate immune function that together will be of interest to a wide community of scientists, clinicians, veterinarians and public health professionals

The threat posed by emerging infectious disease to human health and the economy is substantial. It is important, more now than ever, that we better understand these diseases so that we can develop new treatments and vaccines. On a global level, one of the most important groups of infectious diseases are those that are spread by biting mosquitoes. This includes diseases caused by viruses, such as dengue, zika and chikungunya. Most such infections are found in warm climates. However, due to the worsening climate emergency and increasing globalisation, the areas affected by these diseases has increased at an alarming rate. Today, over one third of the global population now live in areas at risk of outbreaks. The timing and emergence of these epidemics is almost impossible to predict. We suggest that one aspect that can increase our preparation for such outbreaks is a better understanding by which our body defends themselves against virus. This will enable us to design new therapies that are useful against a broad range of these viruses.

Infection with mosquito-borne viruses most often causes a debilitating flu-like illness, which can be associated with severe complications and sometimes even death. This combined with a lack of anti-viral medicines makes it difficult for clinicians to treat and manage patients. In addition, symptoms during the early stages of diseases are similar, irrespective of the virus they are infected with, making a timely diagnosis difficult. Therefore, new medicines that target common aspects of infection, irrespective of the causative virus, have the potential to transform the treatment of patients.

This proposal aims to ultimately reduce the burden of these diseases by increasing our understanding of the skin’s immune defence to infection at the mosquito bite. When mosquitoes bite people they inject virus into the skin. This is a key stage during which the virus infects cells in the skin and replicates, and is common to all mosquito-borne virus infections. Following infection at the bite, the body’s immune response is activated, which if sufficiently robust can hinder the virus from replicating and causing disease. We have recently found that the strength and type of immune responses activated at the mosquito bite can influence how bad the infection becomes in the rest of the body. In particular, we found that a component of immune responses, interferon, is too slow and not sufficiently robust at the mosquito bite to stop the virus. This allows the virus to replicate and spread around the body and cause disease. It is not clear why interferon is not being activated properly. Interestingly, we have recently shown that a skin-applied cream that contains an interferon booster, can dramatically increase the skin’s resistance to virus infection. This showed that it is possible to therapeutically activate the body’s own immune responses at the mosquito bite to alter susceptibility to a wide spectrum of these diseases.

How the interferon response to virus is coordinated by the body at mosquito bites is not well understood. If we want to better design new therapies, we need to know how these immune responses in the skin are organized at the cellular and molecular level. This project will bring together distinct scientific and clinical expertise to transform our understanding immune defence at mosquito bites. In doing so, we will identify new ways to treat and prevent infection.

The role that mosquito derived salivary factors have in modulating infection at the mosquito bite is also not well defined. We will specifically define what role mosquito saliva has in modulating host susceptibility to virus infection. We have already identified one such mosquito salivary factor that increases blood vessel permeability and thereby enhances skin infection with virus. In this project, we define the mechanism behind this observation and additionally seek to define other mosquito derived salivary factors that similarly influence virus infection. Identification of these factors may enable the development of vaccines that target them.

As such, this work has the potential for publication in the very highest impact multidisciplinary journals. We also believe this work is likely to lead to productive new collaborations with industrial and clinical groups that will translate our basic science discoveries into real world impact.

The overall benefit will be to enhance our understanding of immune and inflammatory responses, although where possible we will make use of these findings as best we can by working with pharmaceutical companies and clinicians to develop new medicines and/or strategies to help treat these diseases.

Thus, this project will combine expertise from the distinct disciplines of immunology, virology and dermatology in an imaginative and complementary manner, the results of which will likely benefit a range of academic and clinical disciplines. Within the academic sphere, this work will generate significant and important new immunology and virology insights at a key, but understudied, stage of infection with an increasingly important group of pathogens.

This project provides a unique opportunity to fuse distinct expertise and resources in a novel way that will lead to new academic and translational research opportunities. Thus, by combining the strengths of our ‘underpinning’ animal and human-based studies, with the translational/clinical approaches of our dermatology colleagues, this project will be uniquely placed to both build capacity for future research and in doing so determine the relevance of early skin immune responses on disease severity.

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

Academic and associated beneficiaries

This project will make use of our existing collaborations that have been highly successful in generating key (now published) insights on skin arbovirus infection.

We are ideally placed to now extend this collaborative network to include relevant bioinformatic expertise. Together, this will generate a unique new collaborative relationship that combines expertise

in; dermatology; molecular arbovirology; mosquito biology; and bioinformatics/transcriptome analyses. This will be integrated by our team's existing, complementary expertise in inflammation biology and arbovirus pathogenesis to generate insights of interest to wide number of disciplines. Additionally, our team includes a new PhD student who soon begins a PhD project studying skin patient (psoriasis and eczema) susceptibility to arbovirus. These studies will gain considerable advantage and synergy from the work supported in this proposal, including use of human skin biopsies for explant infection.

In summary, due to the broad-based nature of the proposed work, the wider academic beneficiaries will include a spectrum of virologists, immunologists, translational medical dermatology and infectious disease clinicians.

Longer term non-academic beneficiaries

Our work is highly translational and has great potential for informing the design of new strategies for alleviating the health burden of e.g. mosquito-borne viruses. Based on the findings of our work, we anticipate working with colleagues in clinical medicine and industry to formulate new approaches for the design of medicines and vaccines.

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

This collaborative project will maximise the benefit of work to the institutes and individual research groups involved in several distinct ways;

• there will be expertise transfer between our groups in the areas described above and capability building between them. Dermatology-based academics will be provided with the opportunity to gain expertise in basic biology-based approaches, including in vivo models of infectious disease. In addition, our group will gain access to the clinical and patient resources of our dermatology colleagues.

• as such, this collaboration will provide opportunities for researchers and students in each group to gain new research experiences and skills that will facilitate and enhance their research career development

• this project will also allow our clinical co-investigators to widen the scope of their research portfolios to now also include studying infectious diseases of global importance that particularly affect low and middle-income countries.

We expect to publish the results of this work as several publications in internationally-leading multidisciplinary journals, and additionally, present this work at national and international conferences. To increase the efficiency by which we disseminate our findings through these routes we will also pre-publish our data on BioRxiv. Using this real time feedback approach, we can rapidly disseminate our key findings to the international community. We also have a successful track record of engaging the academic and wider scientific community through a combination of social media. Explaining the importance of scientific research to the wider society is also necessary if we are to continue receiving public support for our research and because we want to promote science as a rewarding career. Public engagement requires adequate and sustained discourse that is communicated in manner appropriate for the target audience. We will target non-specialist audiences using a combination of; online social

media; coverage in traditional media outlets; our website and through face-to-face interaction in both the laboratory (e.g. with visiting school students); and in public environments.

Species and numbers of animals expected to be used

- Mice: Our studies exclusively use mice and we anticipate using up to 5,000 mice over the 5-year timeframe of this project. Note that 1200 mice from protocol 1 will be transferred (in continuous use) in other protocols, so that the total mice used will not exceed 5,000. 300 of the mice in protocol 1 will be used as breeders to maintain transgenic lines.

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.

The mouse is the species of choice as it can be genetically manipulated to alter gene function in ways that are not currently possible using other mammalian species. In addition numerous reagents are available for examining, and intervening in, immune and inflammatory responses in mouse models. We will study the responses of young adult mice as mice at this age best represent human responses.

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

The primary purpose of this project is to understand the body's response to infection with viruses and the inflammation this causes. Therefore, mice will be injected with virus in the skin, along with agents that we hypothesise may influence susceptibility to infection. This will be key for identifying new medicines to treat these diseases. As we are mainly interested in mosquito-borne virus infections, such as Zika virus, we will also allow mosquitoes to bite mice either before virus inoculation. We will use a variety of advanced techniques to describe the course of infection such as imaging and blood sampling. Where possible we will always use techniques that have lowest possible impact on animal welfare.

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

The vast majority of experiments will only result in mild and very transient suffering, such as injection with a needle. We use custom made needles that are exceptionally thin, that we believe cause far less pain than conventional needles. We also use temporary anaesthesia that puts the mice to sleep for a few minutes - this can be done if injection will occur in a sensitive part of the skin such as the foot. Therefore, we minimise any pain or discomfort that may be generated from the injection. If allowed to progress, the virus infection will lead to brain inflammation, encephalitis, which develops very rapidly. This most commonly presents as limb paralysis along with easily detectable sickness behaviour. As soon as these clinical signs of brain infection are detected, the mice will be humanely culled. Therefore,

the total time that the mouse becomes unwell at this later stage of infection will not be more than a few hours.

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 majority of mice (estimated at 90%) will only experience mild suffering, as we will humanely cull the mice prior to the development of more severe disease caused by the virus. However, to study the later stages of diseases and e.g. to determine what effect any new medicines we develop have, we will let some mice progress to more severe disease. The total length of time that a mouse will experience severe suffering will be very brief, as mice tend to develop signs of suffering within a few hours, at which point they will be immediately and humanely culled. This is essential as we can determine whether new medicines are working to stop the disease.

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

- Killed

A retrospective assessment of these predicted harms will be due by 02 August 2026

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 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?

This project will seek to understand the complex biological interaction between biting mosquitoes, the viruses that they carry and their mammalian hosts. We have evidence that mosquito bite inflammation is highly counterproductive and helps viruses establish infection in the skin. This project will work out how the immune system responds to mosquito bites and how viruses spread from bite sites to the blood and other tissues. This will be useful to scientists, health professionals, drug companies and policy makers who shape our response to these epidemics.

Because of their complexity, the immune and inflammatory responses to infection at mosquito bites, it is necessary for us to use mouse models. This is because immune responses involving numerous different cell types and molecules in ways that cannot be yet replicated outside of an animal. This is because immune responses are carefully orchestrated in an intact animal in ways that cannot be recapitulated using non-animal alternatives.

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

We will be employing and using a range of non-animal alternatives that include in vitro cell culture. This includes culture of skin cells, leukocytes and endothelial cells alone and in co-culture to better mimic the microenvironment of the skin.

Why were they not suitable?

These non-animal approaches will help us to reduce the number of mice used considerably. However, if we are to prove that a particular molecule or cell is important and can be targeted therapeutically, we must also undertake some experiments using mice.

A retrospective assessment of replacement will be due by 02 August 2026

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 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?

The more variation there is in the data generated by an experiment, the more animals are required to statistically prove or disprove a result. Based on our published studies we have a good idea of the variation we expect to see in our results. Using this variation, we are able to use statistical methods to calculate how many mice we would need in future experiments. This was undertaken with the help from professional statisticians.

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

We have many years' experience of working with animal experimentation and have developed robust protocols involving the minimum use of animals required to provide statistically significant analysis.

We also obtain advice from statistical analysis colleagues regarding the design of new experiments.

We have also made use of the Experimental Design Assistant (EDA). This is a free online tool from the NC3Rs, designed to guide researchers through the design of their experiments, helping to ensure that they use the minimum number of animals consistent with their scientific objectives, methods to reduce subjective bias, and appropriate statistical analysis.

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

At all times mice will be bred in the most efficient manner possible, with regular communication between the animal welfare officers, the animal house technicians, the personal licence holder and the project licence holder. Regular team meetings once a week will include a dedicated section that will discuss the optimum use of animals. We have devised creative and resourceful ways to maximise the use of animals for example suing multiple tissue from one animal for different projects - such as using bone marrow to generate macrophage cultures, while also using the skin for other experiments. Furthermore, where necessary we will undertake pilot studies that use only a very few mice. This will generate data that can be used to predict what numbers of mice would be required to prove or disprove the hypothesis.

In this way we can greatly reduce the number of animals used.

A retrospective assessment of reduction will be due by 02 August 2026

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

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 be infecting mice with viruses that cause great harm to human health on a global scale. It is inevitable that this will cause some suffering to the mice. The severity of the procedures used will be kept to a minimum, whilst providing meaningful data for translation of these approaches to patient care. For example, we will prevent the majority (more than 90%) of these mice from experiencing anything more than a limited and mild form of suffering. This might include e.g. a needle inoculation. Suffering

will be limited by humanely culling the mice at an early stage of infection, at which point the mice do not perceive any pain or discomfort from the infection.

For some mice, to better understand how these viruses cause disease and how new medicines could treat these diseases, we will let ust a few of the mice progress to later stage of infection. This will cause suffering of the mice to be more than mild, but will be short in duration. This is because we will use a virus that only causes suffering for just a short few hours. Regular monitoring of mice will ensure that mice are immediately and humanely culled to prevent unnecessary suffering. By doing this we will help uncover important new insights into disease and new medicine to help treat them.

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

The mouse is the species of choice and it can be genetically manipulated to alter gene function in ways that are not currently possible using other mammalian species. In addition numerous reagents are available for examining, and intervening in, immune and inflammatory responses in mouse models. Furthermore, mice constitute the 'lowest' mammal recognised to be relevant for immune and inflammatory disease studies.

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

To minimise harm to animals, especially those on procedures with which we have less experience, animals will be monitored regularly for routine signs of ill health or distress. Anaesthetics will be used as appropriate to the procedure being undertaken and advice from local veterinary surgeons will be sought in any situation where animals are showing unpredictable signs of ill health or suffering.

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

Discomfort and distress experienced by the animals will be limited to unavoidable procedures required for the conduct of sound research.

General Guidelines

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NCRs) website (https://nc3rs.org.uk//results-search/all/refinement) is a rich resource for obtaining new approaches for refining animal-based research, with regular updates and evidenced guidance supplied. This published online resource will be consulted with in a continual manner to ensure our experiments are undertaken in an optimally refined manner. The NCR have published guidance that we will use to guide our studies;

Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/laban.1217

In addition, general guidelines on how to conduct experiments in the most refined way, has been published by the NC3Rs in collaboration with the MRC and other funding bodies. This document provides general guidance to researchers and associated veterinary and animal care staff using vertebrates in bioscience research.

https://www.nc3rs.org.uk/sites/default/files/Responsibility%20in%20the%20use%20of%20animals%20in%20bioscience%20research%20-%20July%202015.pdf

Experiment-specific guidance

More specific criteria for the experiments described in this project proposal can be found in our previous publications including

Pan-viral protection against arboviruses by activating skin macrophages at the inoculation site (2020) Bryden SR, Pingen M, Lefteri DA, Miltenburg J, Delang L, Jacobs S, Abdelnabi R, Neyts J, Pondeville E, Major J, Müller M, Khalid H, Tuplin A, Varjak M, Merits A, Edgar J, Graham GJ, Shams K, McKimmie CS. Science Translational Medicine. 12:527. Host

Inflammatory Response to Mosquito Bites Enhances the Severity of Arbovirus Infection (2016) Pingen M, Bryden SR, Pondeville E, Schnettler E, Kohl A, Merits A, Fazakerley JK, Graham GJ, McKimmie CS. Immunity. 44, 1455–1469.

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

We will stay informed in all aspects of the 3Rs using a number of local, national and international resources and practices. Locally, this includes discussing the principles and practice of 3R at dedicated sessions during our weekly team meetings. This will be supplemented with monthly discussion at our local animal house meetings, during which all users can participate and engage in new practices that aim to enhance our procedures and promote the 3Rs. There are also regular postings by email from our institute, in which opportunities to gain training in 3Rs is made available. These will also be promoted i our team meetings. National and international resources will also inform our experimental strategy, with the ultimate aim of employing the principles of 3Rs wherever possible. Such resources include the website for the NC3R. The NCR3 is a UK-based scientific organisation dedicated to replacing, refining and reducing the use of animals in research and testing. Their website provides an extensive library of 3Rs resources. This includes guidelines, practical information and themed hubs. Links to publications, other online resources, and video and training materials are also provided and cab found at; https://www.nc3rs.org.uk/3rs-resources

A retrospective assessment of refinement will be due by 02 August 2026

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?