Home Office

NON-TECHNICAL SUMMARY

Understanding how climate change affects mammalian immunity and control of infection

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Infection, Immunity, Heat, Mammals, Climate crisis

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

To understand the effect of short term (heat wave) and long term ambient temperature differences on mammalian immune function and its response to infection.

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?

Global warming is a reality of major concern. Currently, the global average temperature has increased by 1^{o}C as compared to pre-industrial times, with clear effects on entire ecosystems, flooding and natural disasters. Worryingly, by 2050, global temperatures are predicted to have increased by 2-5^{o}C, exacerbating already visible consequences from global warming. In addition we are seeing an alarming increase in heat waves were temperatures are between 8 and 11^{o}C higher than average temperatures. Rising temperatures impact many aspects of animal life and health, and have lately been shown to severely weaken an animal's ability to fight off infections. If our body's immune system (our defence mechanism against bacteria, viruses and other bugs) cannot fight infections properly, the body becomes vulnerable, leading to severe illness or even death. Thus, global warming could make humans more vulnerable to dying from infections such as the flu. This is particularly concerning as rising temperatures also affect how bugs behave, allowing them to become more dangerous to the body or to spread into new regions. Understanding how higher temperatures weaken the immune system is crucial to design strategies that will help prevent humans from becoming more vulnerable to infections.

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

We will understand how a rise in temperature affects the immune system and subsequently what impact this may have on our ability to control infections. This will be in the context of acute 'heat wave' type increases in temperature or prolonged exposure to smaller temperature changes of about 2-3^{o}C, mimicking global warming. Thus, we will gather new information in this project, which will be published for the scientific, medical and political communities and support future research opportunities.

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

In this study, we aim to elucidate how higher temperature can change the ability to defend the body against infections. Understanding the mechanism behind these changes will give an insight into how we may be able to prevent or treat reduced ability to fight infections due to higher temperatures. From the results of our mouse studies, we can get an insight into how humans may react to similar temperature stress. As well as adding to our general knowledge about the effect of global warming on the human body, our data may enable scientists, doctors and politicians to create strategies that help avoid or treat decreased ability to fight infections due to higher temperatures. This could include strategies to improve the effect of vaccinations or the treatment of an infectious disease during a long hot period, especially in developing countries where people cannot always shield from the heat in climate controlled buildings. Finally, demonstrating that global warming will make our immune systems weaker can be used as yet another argument to advocate for policies to combat climate change.

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: 9700

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 how global warming affects our body's ability to fight infection, 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. In fact 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, would allow us to later look into the role certain genes and inheritance plays when it comes to vulnerability after being exposed to high temperatures.

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

Animals in this project will typically be subjected to increased ambient (meaning 'outside') temperatures for a period of time and then infected with a bug (virus / bacterium) or vaccinated. This will involve either an injection, such as intravenously (i.e into a vein) or intranasal (i.e into the nose) and oral gavage (a tube inserted via the mouth into the stomach). 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. At the end of the experiment all mice will either be humanely killed, or blood collected under deep, terminal anaesthetic where they will be asleep/unconsciousness throughout. The experiment will be typically 1 month long. Some long-term breeding under increased temperature will occur over multiple generations. These animals will then be treated as above.

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 animals at standard room temperature 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.

With increased ambient temperature exposure, 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 are fleeting and should last no more than 24 hours. These mice are not expected to show any long term adverse effects.

Keeping animals at temperatures over 30 degrees could increase incidences of poor lactation in breeding females and heighten aggression in males.

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 40%

Moderate 60%

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?

As part of the work in this program we will use pilot studies to inform us on the control experimental conditions we need to use. These will indicate if we need to alter food intake in control animals, as mice at higher temperature don't eat as much food and if mice at thermoneutrality (29^{o}C) have the same base line outputs as mice at room temperature (21^{o}C) and therefore which mice should be used as controls. We have used the infections in this licence as part of a large screening programme over the last 15 years. The models are set up in a way that allows us to identify differences in test conditions (be that individual gene deleted mice or in this case increased temperature).

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. The data generated in these programs will also inform the work herein. Samples collected from any mice as part of experiments planned will be stored long term at -70^{o}C. 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 increase temperatures.

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.

Litters from breeders at higher temperature will be monitored closely for development as temperature may affect lactation in mothers. Any evidence in pups not developing or litter losses will be discussed with NVS and appropriate actions taken.

All animals 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.