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NON-TECHNICAL SUMMARY

Genetic and external influences on regulation of the immune system

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

immune system, infection, inflammation, radiotherapy

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

We aim to understand how the immune system functions to maintain health by protecting us from infection and damage, but how this sometimes goes wrong and causes disease.

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?

When our body becomes damaged, it is crucial that our immune system responds quickly and appropriately to keep us healthy. An example of such damage is when we are infected by a harmful pathogen like a bacterium or virus. Another example is when we receive treatment for a disease that is useful but can have side-effects- such as when we receive radiotherapy treatment to kill cancer cells but some of our non-cancer cells also get killed. Understanding how our immune system responds to such damage, and how it can malfunction to either fail to deal with infection or cause harmful effects to the damage, is crucial in designing new therapies that help our bodies protect itself from external threats.

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

Outputs will include:

1. A better understanding of how the immune system responds to infection with different types of pathogen

2. A better understanding of how radiation therapy affects the immune system in normal tissue to cause potential side effects

3. Publications that will inform the work of other scientists around the world

4. Development of potential novel targets for therapy for the benefit of the general public

5. Dissemination of understanding to the general public, for example via school visits and public engagement events

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

In the short- to mid-term (1-3 years), the research will aim to benefit other scientists in the field by providing important results that we can publish in scientific journals that inform the research of others.

In the mid- to long-term (3-5 years) the research will aim to benefit patients by providing new targets for disease therapies- for example, ways of treating patients to promote the immune response to infection, or ways of reducing the side-effects of radiation therapy in cancer patients. The eventual

hope is that these findings will therefore be passed on to the general public in the form of new medicines.

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

We aim to publish all research findings from these studies in peer-reviewed journals to inform the research of other scientists worldwide. We will aim to publish negative as well as positive data, to ensure that any approaches that do not result in positive results are not repeated by other scientists.

We will also publicise the findings of the research to a wider audience. For example, during the period of the last Home Office Licence, I took part in an event called 'Pint of Science', where I engaged members of the public in the pub about the specific research we do, including the importance of animal studies, and the potential impact that these studies have for human health.

Species and numbers of animals expected to be used

- Mice: 10000 in protocol 1 (breeding protocol) 13000 in experimental protocols

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.

We use mice in our studies, as they have proven over many decades to be an incredibly valuable model of the human immune system. The human immune system is very complex, with interactions between different types of immune cells within different tissues determining the overall response to infection and other external stimuli. It is therefore unfortunately not possible to use isolated cells, or other lower organisms to model complex immune responses.

Specifically for this project, mouse models have proven extremely valuable in determining responses to infection with different pathogens, such as viruses, bacteria and parasites, as the responses to these pathogens mirror very well the responses seen in humans. Additionally, mice are proving an invaluable model to determine how our tissues respond to other external threats, such as radiation therapy that we study in our project. Radiation therapy is a critical treatment for cancer, but causes short- and longer-term side-effects, which are believed to be driven by the immune system. Mouse models of radiotherapy therefore allow us to dissect the involvement of complex immune interactions in causing such side-effects.

We will generally use adult mice in our experiments (>6-8 weeks of age), as at this age the mouse's immune system has developed to a degree that models the adult human immune system. However, important data suggest that modulation of the immune system in pregnancy and nursing can alter the susceptibility of offspring to external insults such as infection. Therefore, in some experiments we will treat pregnant and nursing mice with substances that alter their immune system, and then

subsequently infect their offspring to try and uncover important ways in which alterations in the immune system during pregnancy and early life can have important effects on the immune system of offspring.

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

There are two main procedures that the animals will be subjected to during the project, which are both key external stimuli that alter our immune system and can have detrimental effects on our health: infection and radiotherapy. The ultimate aim is to determine ways in which to improve the outcome of infection and radiotherapy.

We will use a number of different infectious pathogens to determine important immune cells and molecules that regulate responses to infection. These are viruses, parasites and bacteria. These pathogens will be administered, in the majority of circumstances, via their natural infection route- either via the nasal passages for respiratory pathogens, or via the oral route for oral pathogens.

We will also re-infect mice after they have recovered from their first infection. Sometimes this will be with the same type of pathogen, to determine how so-called 'immunological memory' is formed- a vital cornerstone in how vaccinations work to keep us healthy. In other scenarios we will re-infect with a different type of pathogen, to see if the initial infection makes it more or less likely that the immune system deals well with the subsequent challenge.

In other separate experiments, we will treat mice with radiation, to mirror radiotherapy given to cancer patients. Although extremely important in helping treat cancer, radiotherapy can cause short-term and longer-term damage and side effects in normal tissue, which is believed to be caused by our immune system. Our experiments will involve giving mice a very targeted dose of radiotherapy to either their intestinal or lung region, and attempt to determine how the immune system drives these short and long-term side effects.

In >95% of all experiments, we will analyse the tissues and cells of mice via post-mortem analysis. In <5% of experiments, we will take blood samples during the experiment to monitor the immune system, before analysis of immune cells in tissues by post-mortem at the end of the experiment.

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

What happens to the mouse after infection will depend on the specific type of pathogen they have been infected with.

A single infection with viruses or intestinal bacteria can lead to transient inflammation and weight loss. Weight loss typically appears gradually during the first week of infection. At the peak of illness mice may show a hunched posture and reduced mobility, but in mice where the immune system is not altered in any way, 100% of mice are expected to recover.

A single infection with an intestinal parasite typically leads to no specific adverse effects being observed (e.g. infection with *Trichuris muris*). Infection with some other intestinal parasites (e.g. *Trichniella spiralis, Toxoplasma gondii*) typically leads to intestinal inflammation and weight loss, similar to viral or bacterial infection mentioned above. As above, in this case mice where the immune system is not altered in any way are all expected to recover.

We will also re-infect mice that have been previously infected, but only after full recovery from initial infection. The outcome of the mice will depend on which pathogen they are re-infected with; if this is the same pathogen that they were initial infected with, they will typically have generated so-called ‘immunological memory’, so their immune system will likely deal with the infection more quickly and efficiently, reducing symptoms seen. If the mice are infected with a different pathogen, our hypothesis is that, at least in some circumstances, this will cause an improvement in symptoms due to the body having adapted to the previous infection.

We will also subject some mice to either a single or fractionated dose of radiation, specifically targeted to the intestinal or lung region. Of note, we will not irradiate mice that have previously been infected. This targeted radiotherapy (at the highest dose we will use) typically causes weight loss from days 3-4 post-treatment, peaking at days 5-7, before recovery to previous body weight by days 9-10 post-treatment. We will also monitor mice to determine longer-term side effects of radiotherapy, which may result in some further weight loss but this is typically very infrequent (<5% of mice).

In all of the above experiments, we will have some mice that are not infected or subjected to infection or radiotherapy as a control, which will not experience any weight loss or other symptoms. We will also have other mice that do receive infection or radiotherapy, but also treatment with control substances or substances that lead to modulation of the immune system. We estimate that approximately ~60-70% of mice used in the licence are expected to develop symptoms described above. Transient pain may occur on injection of substances/cells, though in some instances they are anaesthetised when this procedure occurs.

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)?

This project works to a maximum severity of moderate. With uninfected/untreated controls, and the use of therapeutics that aim to reduce symptoms of infection or radiotherapy, we estimate that ~60-70% of the mice undergoing these procedures will reach the moderate severity limit, with the remaining 30-40% staying below this limit.

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

- Killed
- Used in other projects

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?

External threats such as infection and radiotherapy can cause serious health issues in humans, and this is a result of complex interactions between many different types of cells in the body in complex organ systems. We know from our and others previous work that complex interactions between many different immune cells, and also the structural components of the organs, are vital in regulating the immune system. Thus, although it is possible to culture immune cells cultured in the laboratory (e.g. cell lines or cells obtained from mice or humans), such experiments do not take into account blood and air flow, the impact of the nervous system, nor interactions with non-immune cells in the context of complex organ systems. We will use cells and so-called organoids from mice (organoids being tissue isolated from mice and grown in the laboratory) to partially replace mice for pre-screening and hypothesis testing, but unfortunately there are no suitable full replacements for animal models currently.

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

Models considered were:

Cell lines

Primary cells isolated from humans

3D organoid cultures

Primary human tissue

Why were they not suitable?

Cell lines: There are cell lines that were originally derived from different types of immune cells. However, these are often very much altered from the immune cell types found *in vivo*, and cannot model the complex interactions between different immune cells and non-immune cells in organs systems. These complex interactions are crucial in the overall outcome of infection and radiotherapy, which are required to determine whether specific treatments may improve outcome.

Primary cells isolated from humans: An improvement on the use of cell lines, in terms of cells reflecting the actual cells found in humans, are cells isolated from the blood or tissues from humans. However, this approach still comes with the issue that they cannot be used to mirror the complex interactions that are crucial in the overall outcome of infection and radiotherapy, and which are required to determine whether specific treatments may improve outcome.

3D organoid cultures: In recent years, models have been developed that use tissues from humans to produce a 3D structure, or so-called organoid, aimed at mirroring a human organ, and that can be seeded with different cells in an attempt to recapitulate the complex environment of an organ. However, unfortunately such organoids are still limited in their complexity as only limited immune cells can be seeded into them and their make-up only models limited aspects of a real organ (e.g. no air or blood flow like a real lung, no movement of food and waste and no complex microbiome like a real intestine). Additionally, such organoids do not model the complex inter-play between organs that data has shown is extremely important to overall regulation of the immune system.

Primary human tissue: These tissues are very useful for working on human immune cells that have come from a complex environment. However, as with the above examples, major weaknesses include having to isolate cells and work on them outside their complex environment, and there is no ability to study the complex interplay between different organ systems and structures. An additional difficultly is the ability to obtain enough samples from individuals to build up a timeline of outcomes to infection/radiotherapy to interrogate mechanisms of action of the immune system. Obtaining such samples is not possible practically or ethically.

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?

There is an extensive literature using *in vivo* models of respiratory and intestinal pathogen infection, which we have used for over 10 years over two previous project licences. We have been carrying out radiotherapy models with collaborators for the past 4 years, with our collaborators having over 2 decades of experience using such models. Based on these experiences and working with expert biostatisticians, we have estimated numbers in all protocols based on using 4-6 mice per group, which have previously provided strong statistical power and mitigated the need for multiple repeats. Data collected in preliminary experiments will be used to compute the sample size needed in follow up studies.

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

1) Sharing of mice for experiments: We are a large community of immunology-focussed researchers, many of whom use mouse models in their projects. There is overlap in some of the infection and radiotherapy models we all use, thus with correct timing and local communication, multiple scientists can obtain tissue and cells from mice undergoing a particular procedure. This has been ongoing during both of my previous project licences, and will continue in the current one to reduce the numbers used.

2) Confidence in reproducibility due to experience with models: During the past decade I have held a project licence, we have become experienced with knowing what variability and practical considerations are for specific models. For example, in some experiments (e.g radiotherapy experiments) the delivery of radiotherapy is machine-driven and very accurate both in targeting and dose. Therefore we generally use ~4 mice per group for these experiments knowing that variation is not a major issue. In some infection experiments, such as respiratory infections, delivery of the intranasal dose is somewhat more technically challenging, and variation is somewhat higher. Thus, we

regularly use 5-6 mice per group here, knowing that any smaller groups would likely lead to non-significant results.

3) Use of available online resources: The NC3Rs provides extensive online resources to consider a Null (H0), or alternative (H1), hypothesis that enables the correct number of inter- and intra-experimental repeats to be calculated. For new experiments we will use these resources with the aim of reducing numbers used.

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

As in our previous two project licences, we will reduce mice used by sharing tissues with other groups. For example, our local collaborators also employ models of lung viral infection, and our groups communicate to ensure that we share animals as much as possible. Similarly, other local collaborators employ intestinal parasite infection models and we regularly communicate with their team about potential for shared mouse use.

During the previous project licence, in consultation with the local animal staff, we have adopted procedures to more closely monitor breeding and make breeding more efficient (e.g. keep stock male and female cages that can then be bred if needed rather than constant ‘tick-over’ cages that produce litters continuously). We will continue these procedures going forward in this licence.

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 only employ infection and radiotherapy models that have been refined over the years to minimise animal distress and replicate the human scenario better. Procedures involve administration of virus, bacteria and parasites typically by inhalation or oral gavage, targeted radiotherapy using the Small Animal Radiation Research Platform (SARRP) and injection of immune modulators aimed at reducing disease severity.

We constantly strive to reduce any animal suffering experienced. We closely monitor mouse condition (e.g. body weight, mobility, fur condition, body condition score) very regularly during procedures-specific monitoring depending on the specific procedure. At times where our previous experience have shown us that peak symptoms occur, we increase the frequency of monitoring. When anaesthetic is required for administration of substances (e.g. respiratory infection), we typically use inhalational, which allows recovery within a few minutes and prevents mice from losing body temperature whilst immobile.

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

Unfortunately we cannot use less sentient animals for our experiments. Although animal such as Drosophila and zebrafish do have an immune system, this is a lot less complex than the mammalian immune system and so cannot respond in the same way to infectious and radiation challenges that we use in our project. Additionally, the pathogens used in the project- used to model closely human challenges- cannot be used to infect these less sentinel animals.

We cannot use terminally anaesthetised animals as we require the mice to develop immune responses to the external challenges, and to analyse the outcomes of these challenges over time and in response to therapies.

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

During the previous two project licences we have continuously refined procedures, mainly at the level of monitoring of mice to ensure minimisation of harm at all stages. In all experiments, if we detect any signs of unexpected symptoms we will increase monitoring to at least twice daily, and make a specific note about the experiment for future monitoring purposes.

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

We will follow:

The government animal testing and research: guidance for the regulated community (https://www.gov.uk/guidance/research-and-testing-using-animals)

Morton et al 2001, Refining procedures for the administration of substances; Laboratory Animals, 35, 1-41

The NC3Rs webpage: https://www.nc3rs.org.uk/

Standard Operating Procedures developed with the animal facility and the named veterinary surgeon.

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

We will ensure that all personnel who work under this project license routinely interact with the NC3Rs online resource (https://www.nc3rs.org.uk/). We will have a regular agenda item at group meetings to discuss ongoing animal experiments in the context of the 3Rs, and if there is anything that can be implemented based on recent advances.