Home Office

NON-TECHNICAL SUMMARY

Immuno-regulation during parasitic worm infection

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

Helminths, Immunity, Immunoregulation, Co-infection

Animal types	Life stages
Mice	juvenile, adult, embryo, neonate, pregnant
Rats	adult

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 research is to gain a detailed understanding of the relationship between parasitic worms (helminths), their hosts and their hosts' microbiome (all the bacteria/protozoa/fungi that naturally reside in the intestine). This includes understanding how the host responds to these helminth infections, how the immune response is able, or unable, to control these infections and the complexity and consequence of these responses in the light of other infections. Additionally we aim to understand the effects of treatments such as vaccination, changes in the host intestinal microbiome and changes in host diet on the ability of the host to mount an effective immune response.

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?

It is important to undertake this work as helminths are a neglected but medically and veterinary important group of pathogens that are ubiquitous in man and animals throughout the life-course and across the globe causing considerable ill health and disease. Control measures for these parasites are far from effective and there are no vaccines for use in humans and only a handful for domestic stock/companion animals. The impact of helminths on wider host physiology, associated diseases and pathologies and responses to other pathogens is under appreciated and neglected. Critically, animal models provide an opportunity to discover, define and test the mechanisms controlling immunity and disease that are impossible to perform *in vitro* or in humans and will pave the way for development of new treatments and control measures.

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

The research in this project will generate important new information on how the immune system controls and clears helminth infection or does not control helminth infection and regulates chronic infection. Also, it will have identified how this regulation is impacted by co-infection or changes in diet or in the microbiome. It will also have identified experimental vaccine candidates for some of the helminth infections we study. We will also have generated important new information on molecules that some helminths produce to manipulate the host immune system and we will have assessed their capacity to moderate other inflammatory conditions such as allergy. Direct outputs from the work will be peer-reviewed research articles, dataset resources that will be shared with the research community, and presentations at national and international meetings where we will disseminate our discoveries.

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

This will be of major benefit to researchers and scientists working on immunity to infection particularly helminths but also the broader field of immunity to pathogens and immunoregulation in general. In the short to mid-term it will provide the rationale for developing new therapeutic approaches to controlling helminth infection and managing the consequences of chronic infection. Ultimately this research will be of benefit to the billions of humans currently infected with helminths worldwide.

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

We will publish our data in peer reviewed journals in open access format. We actively collaborate with research groups across the world that enhances the impact our findings and we will continue to grow new collaborations. We will publish negative data and unsuccessful approaches both as online supplemental data and in peer reviewed journals that accept robustly performed and analysed data regardless of impact.

Species and numbers of animals expected to be used

- Mice: 12,500
- Rats: 125

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 major host species and life stage to be used is the adult mouse. The mouse is the most well-defined immunological model system available that has remarkable similarity to the immune system of humans. Moreover, the general physiology of the mouse has multiple similarities to that of humans. Importantly, rodents are naturally infected by the majority of the parasite species used and have counterparts in man. One parasite species used is a natural infection in rats and so these will be used to maintain the life cycle but all experimental infections will be carried out in mice. There are many advantages of working with the mouse, the most important being the ability to manipulate the mouse in order to precisely identify critical components involved in disease processes proving their importance. The availability of an extensive array of tools e.g. antibodies specific for different mouse cell populations to precisely identify them, synthetic mouse proteins to stimulate the immune system and mice with alterations in specific immune genes make the mouse an unrivalled system to study. For studies of the microbiota the availability of mice that have no bacteria or microbes, so called germ-free mice, or mice that have only well-defined bacterial populations provide unique and powerful approaches to investigate the importance of particular microbes in immune responses. There is now extensive literature to show that data generated from mouse studies is applicable to helminth studies in man. Ethically, studies in humans are largely restricted to analyses of peripheral blood cells and will not reflect the responses occurring at the most common site of helminth infection (e.g. the intestine). In addition, varied host genetics, unknown infection exposure history and nutritional variation all compound to make it difficult to achieve immunologically meaningful results from naturally acquired

human infections in nature. In the laboratory, conditions can be precisely controlled, longitudinal studies designed to study immunity to single or multiple infections, informative local immune responses monitored, and comparisons made between animals that expel their parasite burden and those that do not.

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

The general experimental plan will involve the use of wild type or genetically modified animals that will be infected with helminth parasites. This may be one or more infection events depending on the question being investigated. It is noteworthy that naturally, animals and humans are repeatedly infected with helminth parasites (often more than one species) throughout their life course. Experiments will utilise male and female mice where possible. We have already published data on the influence of sex on immune responses to helminth parasites.

Depending on parasite/pathogen, up to two different routes of infection may be used. No more than two parasites/pathogens will infect an animal at any one time. Depending on the parasite/pathogen, the majority of infections will last for between 10 and 35 days. Some, however, will be longer e.g. in the example of “trickle infections” whereby low numbers of parasites will be given weekly for up to ten weeks to mimic the natural infection process.

The experimental design will vary depending on the questions being asked. In addition to infection, animals may be administered compounds that modulate the immune response such as antibodies, cytokines that drive different types of immune responses or immunosuppressants such as cortisone. These will usually be administered by a series of repeated intraperitoneal injections during infection. Animals may be immunised, usually subcutaneously, with antigens together with an adjuvant (a compound used to increase vaccine efficacy), usually on 2-3 occasions (i.e. vaccination), prior to infection. In some experiments germ-free or germ-free mice colonised with defined commensal bacteria will be infected with one species of helminth. In some experiments, mice may be placed on a modified diet (e.g. high fat) for several weeks prior to helminth infection. Cumulative effects of multiple treatments will be minimised and although most treatments are minor, animals will be allowed to recover between them. Following infection at pre-optimised time points, animals will be culled and multiple tissues taken for analysis.

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

Experimental infection with helminths in the models we use is either associated with no discomfort or with mild/transient discomfort most often associated with the infection procedure. For some helminths animals experience discomfort (may be moderate and may experience a small weight loss) for longer e.g. for 2-3 days or up to two weeks and in these cases, animals are closely monitored and given Hydragel/softened food during this period. In experiments where non-parasite infections (e.g. influenza) are used, infection is associated with a predictable and manageable transient weight loss. Mouse condition will be monitored, and mice weighed 1-2 times daily during peak infection (days 3 to days 6-10 post-infection). Outside of peak infection (before weight loss and once mice start to regain weight), mice will be checked and weighed every 1-2 days until day 14 when infection will have been cleared.

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)?

Animals will be routinely monitored during the course of experiments and the severity of disease and level of suffering will be graded according to well-defined scoring systems. Overall, 90% of the animals are expected to experience mild suffering, and up to 10% may experience transient moderate suffering.

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?

The questions we wish to address focus on how the host responds (appropriately or inappropriately) to helminth infection. These parasites are large complex multicellular animals that develop, grow and change their characteristics during the course of infection. As such they are exquisitely adapted to their environment. As yet, there are no tissue culture systems that allow the development of all the life cycle changes that helminths go through and animals are absolutely required to provide the appropriate environment for helminth growth and survival. Also, unlike some parasites (such as malaria), it is not possible to freeze life stages of helminths to maintain the life cycle and thus animals are critical for this. To address the majority of the questions we are asking we need an intact immune system i.e. only present in a complete animal, to precisely define the complex interactions between different cell populations that are activated in infected tissues and which move to lymph glands where they respond and then migrate back to the sites of infection such as the intestinal tract to carry out their protective and regulatory effects. Moreover, infection or immunization at one site of the body can influence host physiology at another site e.g. gut infection can influence brain inflammation. The intestinal microbiota of the host has a major influence on the host immune response. To date this complexity cannot be accurately modelled in tissue culture. There are no well-established models of human helminth infection in animals of lower sentience than mice that can be used to carry out this work.

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

As helminths can only be obtained from infected animals, we are constrained by this fact. We can, however, utilise specialised tissue cultures of host cells and parasites to answer very specific questions about the parasites and/or their interactions with isolated cell populations and/or immune molecules. These approaches can sometimes be helpful in deciding a strategy to subsequently employ in animals. In collaboration with colleagues a completely new tissue culture system (organoids) has been developed for one of the helminths we study. Encouragingly, for the first time, it permits infection by this

species and some limited development of the parasite. The system does allow us to investigate specific interactions between parasite and host intestinal cells where they live and ask questions that we can subsequently take into animals in a more focused manner. It is hoped that as the organoid systems develop further, we can build up the complexity of the system (i.e. adding other cell populations such as immune cells and other supportive cells) which should be more informative. Animals will still be required as a source of cells for the organoid cultures.

Why were they not suitable?

Although the *in vitro* systems allow certain questions to be addressed, this is only in a very specific and limited way. Moreover, the cells and organoid systems still rely on animals as a source of cells. The major limitation, however, is the complexity of the system that we are investigating. Host immune responses are the result of finely tuned multi-tissue responses that involve dynamic activities of cells and molecules from multiple tissues working in co-ordination to mediate effects. They are also influenced by different physiological systems such as the endocrine and nervous system and the microbiome. Moreover, helminths change location, in size, complexity and activity as they progress through development. Using mice, we are able to ask and answer very specific questions, addressing our objectives, within such a complex biological setting.

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 calculated the number of animals to be used based upon an estimation of the experiments that we propose to carry out, the number of repeat experiments required, the number of experimental groups involved in order to generate biologically significant results. This is based upon over twenty-five years' experience and data from the kinds of experiments proposed in addition to published data in the literature for similar experiments.

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

We utilise data from our previous published work to guide our required group size calculations. From extensive historical data we have a good idea of how many animals we will need to use to see significant changes between experimental groups in our experiments. We also use powerful statistical tests to help us determine animal groups sizes. For any new experiments where we do not have historical data we conduct pilot experiments to guide our decision making. We have identified optimum time points for taking tissue samples from the different models used which reduces unnecessary use of animals. We also follow ARRIVE and PREPARE guidelines for reporting of research involving animals,

which outlines appropriate study design (e.g. control groups and sample sizes), how to avoid experimental bias, and the analytical framework for simple and complex experiments.

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

We select the most appropriate mouse strain to carry out our studies, based upon experience and the literature. We use both sexes where we can and record any differences in responses as metadata to examine going forward. We aim to use the most technologically advanced assays to collect the data ensuring optimum accuracy and sensitivity and assess as many relevant tissues as possible in a single animal including tissue archiving. Other tissues are offered to other researchers if they can be usefully used. We carefully manage our breeding colonies using the most efficient breeding strategy to generate the numbers of animals required. Any excess animals are offered to other researchers. Interrupted breeding and cryopreservation of sperm/embryos are used as appropriate to reduce numbers. We take advice from the animal care staff who have expertise in maintaining colonies.

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.

Mice are the most appropriate model to use for our studies. Immunologically, they are the most well characterised defined species with a wealth of tools available to help precisely answer the questions we will be asking (e.g. genetically defined inbred and outbred strains, genetically engineered strains, intervention tools such as antibodies etc.). We also know that the different response phenotypes exhibited by different mouse strains reflects the variation in responses seen in outbred human populations. A small number of rats will be required to maintain one of the species of helminth used.

Our studies focus on helminth parasites, the majority of which are natural infections of wild mice. Thus, they are well adapted to their hosts and provide the most appropriate host species to study response in. In common with humans, helminth infections are naturally chronic in nature and most individuals harbour their parasites with no or minimal overt symptoms, the result of a dynamic immunoregulatory state. As a consequence, morbidity rather than mortality is the major consequence of infection, although some individuals following high levels of infection (e.g. children) or prolonged infections (adults) do suffer from pathological responses of infection.

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

Our research aims to inform on human health. The mouse is the least sentient species with the most appropriate physiology, including the immune system, which is remarkably similar to that of humans.

The parasitic diseases we research are caused by multicellular parasites that, in humans can cause considerable morbidity. We mostly use naturally occurring helminth infections in mice that reflect the major human infecting helminth species. There are no well-established animal models of helminth infection in lower vertebrates or invertebrates that accommodate either the relatedness of parasites or the host immune system.

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

In order to study the regulation of infection and the role of the immune system, it is necessary to infect animals with helminths/pathogens. Helminth parasites evolve to avoid being expelled from the host and control immunopathology that is subsequently generated. This is not perfect but, infections are mostly accompanied by relatively mild/moderate symptoms. Moreover, at certain stages of some helminth infections or in co-infections by non-helminths e.g. viruses or bacteria can cause transient discomfort. As will some of the treatments involving administration of substances or immunisations. We will continue to refine our assessment protocols for animal welfare taking advice from animal care staff and take action e.g. administration of local anaesthesia or analgesia (e.g. emulon before sampling blood from the tail vein) if indicated and provide modified diet e.g Hydragel or softened food to encourage feeding for the short duration of symptoms or weight loss.

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

We will follow NC3Rs, ARRIVE and PREPARE guidance and we will continually assess our experimental design in relation to advances within the immunology and parasitology literature.

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

We strive to identify and implement new experimental advances related to 3Rs in our work through current literature and in-house information via email, regular animal facility 3Rs driven information and training meetings and AWERB awaydays. We use an extensive set of in-house Standard Operating Procedures for animal work that are modified considering new developments related to animal welfare. We get personal formative and formal feedback on the 3Rs from project licence mid-term and retrospective reviews.