Home Office

NON-TECHNICAL SUMMARY

Parasitic helminth infections: mechanisms of immunity and immunoregulation

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

immune response, immune regulation, parasitic helminth, vaccination, immune mechanisms

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

1. To better understand how the genes and cells that are activated following infection with parasitic worms that live in the intestine (intestinal nematode parasites), or following vaccination with molecules derived from these worms, regulate or promote immunity.

2. To develop and define ways to modulate infection and/or damage caused by gut dwelling worms by exploring ways to alter the 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?

Gut dwelling parasitic worms are extraordinarily common and cause ill health in around one quarter of the world's population. The project is important for both humans and animals and aims to understand why some individuals are more likely to become infected with worms than others. Developing treatments for parasites is difficult, due to a poorly defined understanding of how the immune system responds to infection and poorly defined mechanisms of worm infection, development and avoidance of immunity. The project will generate a deeper understanding of how the immune system works following infection by worm parasites. Relatively little is known as to the ways in which the body protects itself following this kind of infection. In the long term, understanding better how the immune system responds or does not respond to parasites will be key to the development of for example vaccines. The ultimate goal is to develop new and better ways to control these sorts of infections.

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

The primary output of this project is to identify the ways by which parasitic worms and/or vaccination with parasite molecules regulate the immune response and the damage associated with infection.

We intend to share our findings with the scientific community and will publish at least three new papers describing novel ways by which gut dwelling worms regulate the immune response or mechanisms by which the host protects itself from infection at the level of cells and molecules. We expect to present our work, in the form of a poster or a talk, at both national and international conferences. In addition we will continue to present our work to the public within public engagement events and science festivals. We will also work closely with the Establishment media office to communicate our research to the general public via press releases as appropriate.

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

Medical and Veterinary benefits: Long-term (chronic) disease is a major global problem. In developing countries chronic infectious disease, particularly caused by parasitic worms has a major effect on health on humans. In domestic animals, infection by gut worms also has a major direct health and economic impact worldwide. Our research focusses mainly on the gut whipworm parasite,

Trichuris. However we have included other worms too, as whipworm is one of several parasitic worms that collectively inflict a bigger global disease burden than HIV, malaria or tuberculosis. Through the identification of the key events and processes that occur during the immune response to gut worms we will inform programmes in the field which strive to control infections and reduce the tissue damage caused by infection.

Product outputs: We will gain insights in to the immune response to infection with gut parasites by defining the mechanisms involved in resistance and susceptibility to infection. Our new discoveries will enable us to develop experimental vaccines. When a decision is made to place the data in the public domain, we will do this in the following ways: (i) publication in peer-reviewed journals in the fields of infectious diseases (ii) presentations at national and international conferences (iii) delivery of public engagement events (iv) press releases.

Who will use the outputs and how: The research involves basic bioscience and preclinical mouse models. Our work will be of interest to other academics working in the field of immunity to infection with different types of pathogens, and to immunologists interested in immune response in the gut. Our findings will also be of interest to clinicians and the veterinary field.

Time scales: we would expect to continue publishing our work in this area annually as part of an ongoing research programme. Equally we would expect to discuss our new unpublished data at national/international conferences annually. It is highly likely that we will have identified up to five new drivers of resistance and susceptibility within a 5 year timeframe. It is highly likely that we will have identified up to three new lead antigens and/or antigenic epitopes for inclusion in experimental vaccines within 5-10 years.

Short term benefits: We expect to generate a comprehensive mechanistic analysis of the immune response to intestinal parasitic worm infection defining key genes, cells and molecules involved in acute and chronic intestinal parasite infection. This will have direct application to our fundamental understanding of immunity to gut parasites and also to our broader understanding of intestinal immunity.

Medium term benefits: We expect our fundamental mechanistic work to allow us to develop our work in the area of experimental vaccination and explore novel immunomodulatory approaches based upon the use of parasite derived molecules. We expect other academic researchers to apply the new knowledge we generate to their own experimental systems to test any new paradigms.

Long term benefits: In the longer term we anticipate that our data will translate to studies on human whipworm and other gastrointestinal roundworm infections of man and animals. Our studies in the long term will inform and advance future control programmes. We thus expect the results of our research to play important roles in the reduction of human and animal parasitic diseases.

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

We will submit our research manuscripts to open access journals and we will deposit manuscripts in BioRxiv, the open access preprint repository for the biological sciences.

Sharing our data sets, where appropriate, at conferences and invited talks will allow researchers to test our findings against other types of parasite infections.

In addition to publishing positive outcomes of our research we also strive to publish data where for example a particular gene deletion has no effect on the outcome of infection as this knowledge is useful.

We also strive to share our methodologies and workflows allowing others to adopt our working patterns if appropriate.

Species and numbers of animals expected to be used

- Mice: 17 500

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.

Our studies focus on the infection of adult laboratory mice with male and female gut dwelling parasitic worms, primarily the parasitic whipworm Trichuris muris (*T. muris*). *T. muris* is the equivalent mouse parasite of *Trichuris trichiura* (*T. trichiura*), the whipworm which infects humans. *T. trichiura* and *T. muris* are virtually identical and therefore the mouse model enables us to develop new therapies to treat *T. trichiura* in humans by understanding better how the infected host responds to the worm, what is needed by the host to get rid of the worm and how the worm tries to dampen down the immune response. We will also infect mice with the rodent hookworm Heligmosomoides polygyrus to model human hookworm infection and *Trichinella spiralis* which can infect humans as well as mice.

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

Mice will receive a parasitic infection. Parasites will be given via the mouth usually on one occasion. Subsequently, mice will be treated with for example with an antibody that will block a key component of the immune system via an intraperitoneal injection (typically 1-2 times a week for three weeks; up to 10ml/kg), and the infection allowed to progress for 35 days when we would normally expect adult stages of the parasite to have developed. During these 35 days some mice will have small volumes of blood withdrawn for their tail veins, with no more than two samples taken in any one week and never exceeding the published guidelines on blood sampling. At day 35 mice will be culled by a Schedule one method and their immune responses analysed.

In other experiments mice will receive one subcutaneous vaccination (up to 5ml/kg) with a parasite derived molecule or molecules in a modern adjuvant for example alum or a virus like particle. Mice will receive a second subcutaneous vaccination for example 10-14 days later prior to infecting orally with a gut dwelling parasite (for example *T. muris*). The infection will be allowed to progress for 35 days when we would normally expect to see adult stage parasites and then the mice will be culled by a Schedule one method to see if the vaccine has protected the mice from the infection.

The collection of bacteria in the gut (the microbiome) has a well-established ability to modify immune responses. In experiments involving “faecal transplants” we will deliver a faecal “slurry” orally to recipient mice to see if we can transfer the immune response seen in the donor mice to the recipient simply by transferring the faeces. Oral delivery (via the mouth - “oral gavage”) is a well-established method for transferring microbiomes between mice and is a mild procedure in itself, causing no more than transient mild stress at the time of delivery.

In some experiments we will need to identify which compartment of the immune system is responsible for, for example, resistance to infection. This can be done by irradiating mice to remove one of the two compartments (the immune cells, leaving the non-immune cells) and then restoring the immune cell compartment via a specific type of bone marrow transfer creating the “bone marrow chimaera”. These mice, once the bone marrow has established, may then go on to have a vaccination and an infection in order to identify which type of cell is important in protection.

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

Mice may experience short term discomfort and stress at the time of the procedure such as the delivery of substances via the mouth (oral gavage). These effects are transient.

Weight loss is expected to occur in the context of some of the treatments. For example Dextran Sulphate will induce colitis and thus weight loss may occur due to diarrhoea/dehydration. In these cases weight loss can be managed by providing wet food ("mash") and usually animals respond within 24 hours. Animals will be closely monitored and weighed daily.

Whole body irradiation and partial body irradiation can have several effects as it destroys proliferating cells including those of the blood system (the hematopoietic system) and intestine. Loss of these cells can lead to diarrhoea, dehydration and infection, possibly leading to death. We will use a split dose over several hours to minimise adverse effects and restrict damage to the haematopoietic and gastrointestinal systems. Animals will be closely monitored during and after irradiation, weighed daily during the first 7 days where transient weight loss can occur, and provided with mash and antibiotics in their drinking water. Any mouse showing distress will be reported to BSF staff or NVS and if necessary humanely killed. Mice given gene inducers or cell depleting substances can show modest weight loss (eg less than 15%) and minimal loss of condition. This has been observed intermittently and is transient and does not extend beyond the period of active dosing. Weight loss may occur if mice are fed a calorie restricted diet. Thus for all dietary interventions body condition and weight will be monitored and if weight loss exceeds 20% mice will be culled by a Schedule 1 method.

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 experimental protocols employed under this licence are classified as moderate. There is one main harmful effect, relating to Irradiation and bone marrow transplantation where all mice will be affected post irradiation, and require daily monitoring until the bone marrow transplant has established. In all other cases, such as administration of parasites and faecal transplants, the proportion of mice adversely affected is expected to be below 0.1%

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?

Our studies are interested in understanding how the host responds (appropriately or inappropriately) to infection, immunisation or during immunological disease. These questions require an in vivo model to precisely define the complex interactions between mixed cell populations that are activated in lymph nodes, respond and then move to the sites of response such as epithelial tissues via specific receptor ligand interactions. To date these cannot be accurately modelled in vitro. In terms of parasitic worm infections, none can be maintained in vitro or indeed infect tissues to mimic the interplay between responses at the infection site and subsequent response in the lymphoid tissue.

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

We are able to use in vitro methodologies that take place outside of an animal in a petri dish, in some cases, to make informed decisions as to how to progress the in vivo work. For example, by exposing cells from infected mice to antigens in vitro we can analyse the key cytokines/chemokine/anti-microbials they secrete. Likewise changes (up or down) in receptor expression both at the mRNA and protein level will enable us to make informed decisions, for instance, as to which molecules we should focus on to work out their function in the infection or disease setting in vivo.

Further, in our analyses of parasite derived molecules that modulate the immune system to date we have made extensive use of cell lines developed from the cells which form our barrier surfaces such as those that line the gut and the skin (epithelial cell lines) and macrophage cell lines to screen parasite secretions for immunomodulatory properties prior to screening them against primary cell lines. This has allowed us to Replace the use of animals where appropriate.

We are aware of the work by colleagues developing a model system where early stages of the parasite can be maintained in a piece of gut tissue outside of the body in a petri dish. This is their in vitro organoid model for live helminths but currently this is only useful for looking at early (larval) stages of infection and cannot be used to explore holistically the host immune response to infection. We will continue to monitor the development of this system in case it becomes suitable as a tool to meet our aims and objectives in the future.

Why were they not suitable?

In vitro methods can help us make informed decisions as to how to progress the in vivo work. However they cannot recapitulate fully the host parasite interactions that occur in vivo. Further the parasites we

work with are still unable to complete their life cycles in vitro and in order to understand the host immune response to infection an animal model (the host) is essential.

Since our current project licence was granted there have been no new non-animal alternatives that have become available that we are aware of. As mentioned above the in vitro organoid model for live helminths is currently only useful for looking at early (larval) stages of infection.

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?

To successfully achieve the objectives of the programme of work it is important that enough animals per time point are used to achieve significant, meaningful results. We have received advice on the proposed experimental design and methods of analysis of the results from statistical experts. We have provided data, for example numbers of parasites and levels of antibodies to show how variable the data sets typically are, and these have been used to calculate the group sizes we will need to use in order to see significant differences where significant differences exist.

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

For in vivo experiments my staff use the NC3Rs Experimental Design Assistant as well as consultation with an external statistical consultant.

Egg doses for infections are carefully monitored over several years to optimise the numbers of healthy worms generated whilst minimising any adverse effects from a high worm burden. *T. muris* does not require continual maintenance in mice with infective eggs stored for years at 4 degrees. Thus to keep the parasite in the laboratory mice need only be infected when there is a need to generate more parasite eggs or parasite derived products.

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

One of our key technical goals is minimisation of variance; this is pivotal in determining the number of animals required to demonstrate biologically relevant differences (supported by statistically significant changes). Steps used to minimise variance within our models include

• Use the same strain of mice, and purchase from a single supplier with minimum variance in weight (16-18g at delivery 22-25g at experiment).

• Use as far as possible the same egg batch to establish parasite infections

• Prepare the substance to be delivered the same way in each experiment.

• Design model endpoints to occur at times when variance is minimal

• Study multiple compounds within one experiment, minimising the number of untreated control groups.

• Use SOPs, to ensure comparability between operators and studies.

• Calculate sample size based on available data before the experiment.

• Use explicit inclusion and exclusion criteria.

• Use randomisation of treatment and controls.

• Where possible we blind treatments; worm burden analyses are always carried out in a blinded fashion.

To increase the efficiency of our mouse breeding colonies where possible we use of male and female mice to both avoid culling surplus stock and to better model the variation experienced in the real world.

We always try to share our mouse colonies with other researchers.

During experiments we harvest and archive multiple tissues from any one animal for future analyses, and where possible we share experiments between researchers asking different questions within the same experimental setup.

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.

The choice of animal model is the laboratory mouse.

Our studies focus on the use of the laboratory mouse. The three gut dwelling worms used in this project are all models of human disease. For example, Trichuris muris in the mouse is a validated model of Trichuris trichiura infection in man. Importantly the mouse species of Trichuris is remarkably similar to the human species of Trichuris at the genetic, antigenic, and physiological levels and also triggers similar immune responses in its host. Thus the mouse model of human trichuriasis enables us to develop new therapies to treat Trichuris trichiura in humans. Heligmosomoides is a rodent

hookworm modelling hookworm infection in humans; and Trichinella spiralis is a worm that infects both humans and mice.

Notably, *T. muris, T. spiralis and H. polygyrus* are all natural parasites of mice and thus have little impact in terms of lasting harm to their host.

Modelling the human disease: The relevance of the mouse model to infection in man is important. In the context of immunity to infection, we, and others have shown that a Type 2 immune response is required for worm elimination in the mouse. Likewise a similar Type 2 associated protective immune responses in man. Although it is possible to study immunity to infection in man the infection status and infection history of the study population is usually undefined and longitudinal studies are difficult. Ethically, studies are restricted to analyses of peripheral blood cells, few of which will be parasite specific effector cells as such cells are stimulated in the local draining mesenteric lymph nodes and then home to the site of infection, the gut. In addition, differences in exposure and diet all compound to make it hard to achieve immunologically meaningful results in the field.

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

Our work focuses on understanding the way a host responds to infection both in terms of immunity to infection and how infections are regulated by the immune system. In both instances we need to study the fully mature adult immune system and thus need to use adult stage mice for our experiments.

T. muris, H. polygyrus and Trichinella spiralis need to live inside the mouse host in order to complete their life cycles. T. muris for example takes 35 days to develop from the infective egg stage through to the adult stage of the parasite and therefore infections cannot be performed under terminal anaesthesia or at an immature life stage.

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

Minimising animal suffering: It is our aim to reduce any excessive suffering or pain experienced by the animals (Refinement) and to apply appropriate humane endpoints (as detailed in the protocols). There is regular close monitoring of all animals by scientific and animal care staff on a daily basis. Many of the experiments will involve only mild intervention protocols and be of a limited duration. We will always adopt the least harmful approach necessary to meet the objectives of the work.

When we combine our vaccine with certain substances, the substance can boost the effects of the vaccine making them stronger. Such substances are call adjuvants. For our vaccination studies we have only used formulations that minimise the risk of any harm. Freund’s complete adjuvant is very good at boosting immune responses. However it is made up in part of bacterial components and can cause side effects such as ulcers at the site of injection. Thus we will exclude the use of Freund’s complete adjuvant and only use less reactive adjuvants typically already licensed for use in man, such as alum. The protocols employed are well established in our lab and designed not to induce suffering in animals.

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

We will follow the PREPARE guidelines (https://norecopa.no/prepare) for all our experimental work and design experiments so we can use ARRIVE guidelines (https://arriveguidelines.org/) for publications. In addition, prior to any animal studies we will prepare and submit a full experimental study plan to the animal unit to ensure all studies are carried out in line with best practices. We will conform to the principles described in the Working Party report "Refining procedures for the administration of substances" in Laboratory Animals (2001) 35, 1-41.

Use of male and female mice: when growing adult stage parasites in vivo we prefer to use male mice as they tolerate the infection better than females. In situations where we need to use female mice to minimise harm we will only use mice above 22g weight.

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

We will stay informed of 3Rs advances through

- NC3Rs newsletters

- animal unit newsletters

- discussions with other in vivo researchers

- seminars put on through the animal facility

Any changes to best practice will be discussed with the NACWO and implemented where appropriate.