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Home Office

NON-TECHNICAL SUMMARY

Mechanisms regulating local and systemic immunity in intestinal health and 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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

immunology, inflammation, infection, gut, Inflammatory bowel diseases (IBD)

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant 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?

This project has two overall aims: 1) Understand the mechanisms that regulate the local immune system in the gut in health and inflammation; and 2) Determine how the gut can communicate to the rest of the body to alter systemic immunity (e.g. immune cells in the blood and spleen).

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 because appropriate control of the local immune system in the gut is crucial to ensure human health. The gut is constantly exposed to a wide variety of harmless factors including food and commensal (good) bacteria but is also a major site of infection by pathogens and parasites (e.g. pathogenic bacteria like Salmonella or parasitic worms). If the immune system inappropriately responds to harmless factors this can lead to unwanted inflammation as occurs in inflammatory bowel diseases (IBD). Reciprocally, if the immune system does not respond quickly enough to infection this can lead to uncontrolled outgrowth of pathogens which can be life-threatening. This project aims to understand the underlying mechanisms that control gut immunity and, therefore, could inform development of drugs that suppress inflammation in IBD or that boost the immune system following infection.

Additionally, it is increasingly appreciated that a healthy gut barrier has implications for systemic health. For example, poorly controlled gut inflammation can lead to leakage of bacteria and their products into circulation resulting in sepsis. Sepsis is a life-threatening condition that can lead to injury to many bodily organs including kidneys, lung and liver. Alongside investigating local control of the gut immune system this project will also investigate the mechanisms that control systemic immunity during gut inflammation. This could inform development of therapies for sepsis.

Specific information about importance of IBD, gut parasite infection and sepsis is given below:

Inflammatory Bowel Diseases: The intestine is a major site of human inflammatory disease, for example inflammatory bowel diseases (IBD). IBD affects 0.5-1% of the UK, affecting approx. 620,000

people with rising incidence. The condition is extremely life limiting and treatment costs per year are high (estimated £1 billion per year to the NHS) – with many ultimately failing current therapies. Additionally, IBD patients are at dramatically increased risk of developing bowel cancer. It is, therefore, crucial that we better understand the immunobiology of IBD.

Intestinal parasite infections: The gut is a dominant site of chronic infection, as occurs during parasite infection. Chronic gut parasite infections are highly prevalent worldwide with over 3.5 billion people estimated to be infected. While gut parasites are not life threatening they can be life limiting and have profound effects on child growth and cognitive development. Although, therapies exist to clear gut parasites they do not lead to immunity and individuals are often rapidly re-infected.

Sepsis: One problem in the context of gut inflammation or gut infection is that individuals are also at increased risk of systemic bacterial infection, as occurs in sepsis. Sepsis is a lifethreatening condition that can lead to severe long-term health complications and affects around 245,000 people in the UK each year. Currently mechanisms to identify patients at high risk of sepsis and to treat sepsis are extremely limited.

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

By the end of this project we expect to see primarily new information and publications relating to mechanisms that underlie gut immunity and how gut immunity is linked to systemic immune responses. Examples of the type of new information that we could discover include: 1) New receptor on gut immune cells that suppresses intestinal inflammation. This information could be shared with our industrial collaborators to facilitate identification of candidate drugs that target the new receptor; and 2) Protein expressed on circulating immune cells that is associated with killing of systemic bacterial infections during gut inflammation. This information could be shared with our clinical collaborators to establish if expression of this protein could be used as a biomarker to identify patients at low or high risk of sepsis.

A secondary benefit to our project would be the development of new or refined methodologies for studying of the gut immune system and the immune system in general. This could have implications for medical researchers working in diverse disease settings. Examples could include: 1) Development of new transgenic animals; or 2) Protocols for optimised use of antibodies that recognise immune cell-derived factors.

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

Scientific Community: This programme of work will generate new data, research tools and advance research methods which will be shared with the scientific community with the ultimate effect of promoting scientific discovery. This impact will occur in both the short term - following publication or talks but, overall support scientific advancement worldwide in the long term. Data generated will benefit researchers who focus on immunology, infection biology and gut inflammatory diseases.

Healthcare Sector: Outputs from this research will, in the longer-term, have the potential to impact the medical healthcare sector. This work will provide basic biological insights into the drivers of gut inflammation and the mechanisms by which this disease impacts systemic immunity. It is hoped our studies will provide novel therapeutic targets for the treatment of gut inflammation, gut infection or sepsis. Our studies could also improve mechanisms to identify groups of patients that could be treated with certain therapies.

Biomedical Industry: Data generated from this project will also support the biomedical industry, as findings from our research are translated into the clinic. This impact will occur in the longer-term as novel therapeutics and/or treatment strategies emerge from our data sets.

General Public: The importance of gut inflammation to overall health is increasingly being recognised. Information obtained from this project can be used to engage the general public in the vital importance of gut health and its relevance to our overall systemic health.

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

Dissemination of new knowledge: Output from this work will be published in highly visible (high-impact) journals which are read by a wide audience. Following publication of data in scientific papers we will make use of social media platforms to further publicise our work, including contacting daily newspapers and magazines (which have run stories on our work in the past). Alongside this, participants undertaking work on this project will be supported to discuss the work widely, presenting seminars and giving talks both locally and globally.

Collaborations: We already have in place a global network of academic and clinical collaborators that we will engage with as we develop this work programme. We also, have strong interactions will clinical collaborators locally through the establishment. Outputs with collaborators will be maximised by regular meetings and data exchanges.

Publication of Negative Data: Although not common we have a strong track-record of publishing negative results. We will continue this whilst undertaking this project, ensuring publication in the most appropriate journals possible.

Species and numbers of animals expected to be used

- Mice: 6500

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 studies outlined will utilise juvenile and adult mice, which may be genetically modified. These life stages are employed to study the precise mechanisms that control gut infection and inflammation. Mice are the most appropriate species for these studies as the systems which we are studying here (gut and systemic immunity – systemic immunity refers mainly to immune responses in blood, spleen, and bone marrow) are well studied and many features are well reproduced between mice and humans.

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

Approximately 75% of the animals utilised on the project will undergo procedures to investigate how gut health is controlled, how this is altered in the context of gut infection (intestinal worm) and how this affects systemic immunity. To achieve this the following approaches will be used:

Gut Infection will be induced in mice. This will be via orally giving larvae of a mouse adapted gut worm. Since this is a mouse adapted gut infection there will be limited obvious effects of inflammation. Usually mice will be infected for 14 days. In some animals we will assess systemic immunity by giving a non-virulent strain of bacteria systemically.

Gut Inflammation will be induced in mice. In around 25% of the animals inflammation may be induced that is more similar to human IBD that will be of moderate severity. This will be undertaken using a chemical (DSS) that causes damage to the gut epithelium similar to that observed in human IBD. Usually mice will be treated for 7 days with DSS.

Prior to, and/or, during these procedures the immune system may be manipulated, through application (typically by injecting or oral administration) of reagents to target specific cell types (e.g. through use of antibodies or tracking agents) or through transfer of cell populations. Additionally, in around 25% of the animals, radiation may be used to generate bone marrow chimeras. This is a bone marrow transplant where bone marrow of a genetically modified animal can be transferred into normal mouse.

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

Animals may experience mild adverse effects such as temporary stress or brief pain and discomfort. Temporary stress may be induced by a brief period of restraint, for example in order to administer an injection.

Weight loss and overt signs of gut inflammation (e.g. bleeding) can occur in the context of DSS-driven inflammation. Additionally, when bone marrow chimeras are generated or mice are challenged with non-virulent bacteria systemically weight loss can occur. In all cases this will typically be in the range of around 10%, with this weight loss being transient, for example ranging from around 72 hrs following systemic bacteria challenge to 3 weeks in bone marrow chimeras.

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 studies outlined in this project will result in the following proportions of animals actually experiencing the following severity ratings:

Sub-threshold – approximately 25%

Mild – approximately 50%

Moderate – approximately 25%

What will happen to animals used in 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?

(1) There are not any viable in vitro or in silico models able to reconstitute the complex interactions occurring between the gut, host immune cells and microbiota and the systemic immune response. The aim of our research is to understand how immune responses are appropriately controlled in the gut, and how failure to control immune responses in the gut can impact systemic immune responses; in vivo models provide a unique window to probe this.

(2) The mouse is a good model to understand the human gut, as the immunological network and tissue structure is similar in mice and man and the important features of gut inflammation are also similar in mouse and man.

(3) Inbred mouse strains will be used, allowing uniformity between animals and experimental repeats.

(4) There is considerable use of knockout (mice that have been genetically altered to lack a specific gene) and transgenic animals (mice that express a genetically changed form of a gene), which are not available for other species. Transgenic mice are already generated, are well characterized and available for use to assess gene and cellular functions.

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

In vitro: Approaches that include cell-lines or co-culture systems (where multiple cell types are cultured together). We utilise such approaches as these i) inform the animal studies to be undertaken and ii) further validate targets and pathways identified in animal models.

Human samples: we have an active research program obtaining and examining systemic immunity in a wide-variety of human inflammatory diseases. With detailed clinical information and patient meta-data such samples can be used to correlate immune phenotype with disease characteristics. Where possible we aim to use integrated data from our murine and human studies to develop In Silico approaches to replace some animal studies in the future.

Neither of these approaches fully replicate the complex environment of the gut or model the communication (e.g. through release of factors into the blood) between the gut and the systemic immune response.

Why were they not suitable?

Although the above approaches are valid platforms for research investigation neither fully replace animal models for the following reasons: i) Our studies in human can only be correlative and will not mechanistically link cause and effect, which can only be achieved in animal models. ii) our research questions require complex in vivo systems to precisely define the complex interactions between heterogeneous cell populations in their tissue microenvironment and also how they may impact responses at sites distal to local intestinal inflammation. To date these cannot be accurately modelled in vitro.

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?

Our group has extensive experience with the methodologies and approaches outlined here and of running projects of a similar scope. Consequently, estimates of animal numbers are based firstly on previous experience with the models to be utilised and the types of data generated, and secondly with careful consideration of the experimental design.

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

Throughout the duration of this licence, we will continue to consider the design of each experiment. As we generate new data sets these will be used to further refine our experimental design.

For this application we have utilised data sets already generated from experiments which have led us to undertake this project. These data sets, along with our research objectives, have been discussed

with the institute statistician, allowing us to get specialist advice on the types of experiments that will be undertaken and the nature of the datasets that will be collected. This has supported development of appropriately designed experiments which will yield biologically relevant and interpretable data with the fewest numbers of animals possible. We will continue to liaise with this expert as our project develops and new data is generated.

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

Throughout this project, optimal animal usage will be achieved by the following means:

1) Utilising the most effective and efficient means to breed transgenic mouse lines. This will be achieved by consulting local and commercial experts. In addition, we employ an electronic stock management tool to monitor our colonies allowing real-time checking and maintenance. In this way we will minimise numbers of animals bred whilst still allowing us to undertake experiments from which we can generate relaible data.

2) Pilot studies will be employed to optimise experimental conditions when we are developing new approaches.

3) We will maximise the amount of data that can be gathered from a single animal by using the latest technologies and most efficient tissue processing methodologies.

4) We will archive all tissue possible from each experiment. We also make our banked tissue available to our collaborators.

5) We will discuss our findings and share data generated with collaborators and the scientific community (for example through depositing large datasets in online data repositories). In this way we will maximise the scientific knowledge than can be obtained from our animal studies.

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.

This project involves the use of experimental infections and models of gut infection and inflammation. We will employ the most refined models available to address our experimental objectives. For example:

For the gastrointestinal (GI) challenges, the models to be used have been selected to drive well-defined immune responses in the gastrointestinal tract, leading to acute inflammation which will resolve, i.e. none of the methods employed lead to the establishment of chronic inflammatory reactions and as such, mouse suffering is kept to a minimum. All GI challenges employed have been well optimised in terms of dosing route, dosage and time post-administration in order to induce a robust and consistent response whilst causing the least harm and suffering to experimental animals.

Where we seek to administer reagents to experimental animals we utilise the most refined route of administration possible. Furthermore we always seek to minimise the numbers of doses of a treatment in order to achieve our objective, this may involve pilot studies to identify an optimal dosing regimen.

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

We cannot replace these studies in mice with studies in less sentient species, such as insects or fish, to achieve our objectives. Such species lack a defined oral barrier which is comparable to humans. Moreover, they lack the complex immune system found in higher order species.

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

1) Appropriate animal handling techniques will be employed and updates, which further reduce stress and discomfort, employed as they are developed.

2) After procedures animals will be closely monitored in line with the refinement controls listed with each experimental step, for example this will involve detailed monitoring of weight and condition. Where transient weight loss is expected following some procedures, soft food/mash will be provided on the cage floor.

3) All animals will have standard environmental enrichment in cages, such as bedding and nesting material, refuges and gnawing sticks and whenever possible, not singly housed.

4) When giving antibiotics in drinking water sweetener will be added to the solution to make more palatable.

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

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.

For specific models, we read papers from other groups doing similar experiments, as well as consulting directly with other researchers to discuss the most refined procedures.

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

Myself, as well as those employed by me and working under this license, will stay informed on recent advances in 3R approaches by staying up to date with NC3Rs recommendations and developments. This information from the NC3Rs is obtained through interaction with their website, local seminars, reading their updates and published bulletins and also through liaising with local 3Rs manager. We also continue to discuss further refinement opportunities with the NVS and NACWO and through interaction with collaborators and the wider scientific community at conferences, workshops and seminars.