Home Office

NON-TECHNICAL SUMMARY

Neutrophil-T cell interactions in inflammatory disease

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

T cells, neutrophils, IL-17, cancer, inflammation

Animal types	Life stages
Mice	embryo, neonate, 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?

To understand the impact of neutrophils on the development of T cell responses, both in healthy physiology and during inflammatory 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?

Neutrophils and T cells are two of the most abundant cell types in humans, and they interact frequently during health and disease. We know from work done by us and by others that this has profound effects on the phenotype and behaviour of the T cells.

Dysregulated T cell responses are the driving force behind a large number of autoimmune conditions, including Multiple Sclerosis, inflammatory bowel disease, rheumatoid arthritis, and psoriasis. These diseases have enormous impacts on patients, their families and the NHS. For example, Multiple Sclerosis (MS) is a focus of the research in this project. MS is a neurodegenerative disease which involves progressive development of symptoms including pain, fatigue, vision problems, and difficulty walking and moving. 1 in every 400-500 people is at risk of MS, and Scotland has the highest rates in the world, for reasons we don't completely understand. Life expectancy is 5-10 years lower for a patient with MS than the general population.

Inflammatory bowel disease (IBD) is another disease which this project will investigate. Rates of IBD are continuing to rise, and it now affects close to 1 in 100 people in the UK, meaning a huge impact on the NHS. This group of diseases (comprising ulcerative colitis and Crohn's disease) has enormous impacts on patients, with symptoms including pain, incontinence, bleeding and anaemia, and severe weight loss. 70% of patients with Crohn's disease will need surgery at some point.

We know that T cells are one of the important factors causing MS and Crohn's disease, as well as other autoimmune diseases. Current therapies for autoimmunity target T cells and have been fairly successful. However, some problems remain. Not all patients respond well to the treatments, and response tends to wane over time, meaning new therapies are always needed. There are often also significant side effects. Blocking all T cells from the circulation, as some therapies do, means patients are very vulnerable to serious infection. There is therefore an unmet clinical need to understand how T cells develop, what interactions they have with other cells, and how they are driven into pathogenic behaviour. This is key to developing novel therapies for autoimmunity and improving the ones we have now. We believe that understanding how T cells interact with neutrophils is an essential part of this

approach. We propose that understanding how T cells interact with neutrophils is an essential part of this approach and it is this aspect of autoimmune disease we focus on.

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

The project will yield a large amount of novel scientific data. This includes a mapping of T cell / neutrophil interactions in healthy physiology and during a number of inflammatory diseases; in-depth analysis of T cell activity, survival and cytokine production in a number of tissues during disease; and a spatial analysis of interactions between the cell types in lymph nodes in particular. This information will be published.

Protocols will be developed and optimised throughout the project. These may relate to *in vivo* work (for example optimum methods for isolating neutrophils from mouse brain tissue) or may be *in vitro* protocols (for example optimised staining panels for confocal microscopy).

Novel GA mice will be generated throughout the five years of the project.

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

The data generated will be published and will be of interest to T cell immunologists, neutrophil biologists, clinicians helping patients with autoimmune diseases, and anyone interested in developing novel therapies to control dysregulated T cell activity. It is likely that immunologists will use the data generated first, with considerable work in patient groups to follow before clinicians will be able to use the information generated. In this way the clinical use may not occur until after the project has completed.

Protocol and method generation, and novel GA mouse strains, will be of immediate benefit to scientists worldwide.

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

All data will be published in peer-reviewed open access journals. Data which does not lead to publication will be released on request, on BioRXiv, or on the lab website. In this way all knowledge gained during this project will be shared with the maximum number of people, whether or not it leads to high-impact publications.

The data will be presented at internal seminars, at other universities, and at national and international conferences. These presentations will be given by all members of the lab team (PI, PhD students, MSc students, and postdoctoral fellows).

All new and optimised protocols will be released on protocols.io or an equivalent free, open-access methods sharing site, as we have done previously. Novel methods will be published in open-access peer-reviewed journals. All protocols and methods will be linked to on the lab website. We welcome anyone to the lab to observe and learn our protocols.

New strains of mice generated will be deposited in the EMMA repository. Live mice will be shared with anyone who requests them.

Species and numbers of animals expected to be used

- Mice: 9750

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 project is informed, in the first instance, by human physiology and disease and we perform a large number of experiments on human cells and tissues. However, understanding the mechanisms underpinning our observations is not yet feasible in human samples. In particular, it is not possible to analyse brain tissue from patients with Multiple Sclerosis until post-mortem, and it is not ethical to repeatedly take cerebrospinal fluid for scientific reasons. In this case therefore, using a mouse model of MS is essential in order to understand what immune responses are occurring at the earliest stages of disease. In other projects, we use many tissues donated by patients but this is often treated in a suboptimal way. For example, tumour tissue is fixed before donation so that it can be examined by a pathologist. This means that *ex vivo* untreated tumour tissue is not available from patients and using mice is the best way to examine it.

In addition, being able to generate GA animals with a gene deleted for particular T cell or neutrophil mediator ensures that mechanisms of disease can be deeply understood. The use of conditional depletion of cells, or transgenic T cells which only respond to one antigen, means we can unravel the mechanisms of disease development at a molecular level.

Mice are the best model animal to use for this project. Their similarity to the human immune system is well understood - in particular, T cells from mice and humans are very similar. Therapeutics currently in use for MS were generated using the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), re-enforcing the utility of mice for these studies. Their genetic tractability and the huge range of resources available for study makes them far superior to rats for this project. In particular, there are enormous numbers of GA mice available to study the immune system. Drosophila or zebrafish, while useful for studying the innate immune response, are not suitable for T cell studies or longer-term studies of chronic disease.

Adult mice will be used in experimental protocols (Protocols 2, 3, 4 and 5). We are interested in understanding how chronic diseases such as MS and solid cancers are triggered in adult patients. As such, using neonate or juvenile mice is not suitable.

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

The typical mouse in this project is a GA mouse bred under Protocol 1, killed by schedule 1 methods, with tissues removed for *in vitro* analysis and culture. This process accounts for more than two thirds of the animals used in our projects. Of the mice that are used in other protocols, we anticipate

approximately one third will be used in protocol 2, one third in protocol 3, and the remaining third will be used in protocols 4, 5 and 6.

For Protocol 2, the typical experience will be a mouse injected with transgenic T cells before being inoculated with antigen in adjuvant. This mouse therefore experiences one intravenous and one subcutaneous injection. These experiments typically last for 7 days during which the mouse is monitored daily. A smaller group of mice may have an immunological mediator such as a cytokine or neutrophil peptide depleted during the experiment, which may involve multiple intraperitoneal injections throughout the experiment timecourse.

For Protocol 3, the typical experience will be a mouse injected with myelin in complete Freund's adjuvant, followed by pertussis toxin, which develops signs of EAE illness. This mouse therefore experiences one subcutaneous and one intraperitoneal injection. These experiments can last up to 28 days but the usual time for a mouse to be killed is on days 10-14. A smaller group of mice may have an immunological mediator such as a cytokine or neutrophil peptide depleted during the experiment, which may involve multiple intraperitoneal injections throughout the experiment timecourse.

For Protocol 4, the most likely experience for the majority of mice is to be injected with tumour cells, and for the tumour size to be monitored over the next 14 days. These mice will therefore experience one subcutaneous injection. A smaller group of mice may have an immunological mediator such as a cytokine or neutrophil peptide depleted during the experiment, which may involve multiple intraperitoneal injections throughout the experiment timecourse.

For Protocol 5, the typical mouse will experience being given DSS in drinking water, after which they will develop signs of colitis. They will typically be killed on day 7 of the experiment. A smaller group of mice may have an immunological mediator such as a cytokine or neutrophil peptide depleted during the experiment, which may involve multiple intraperitoneal injections throughout the experiment timecourse.

For protocol 6, the typical mouse will experience being given an intravenous injection of T cells, after which they will develop signs of colitis. They will typically be killed after 4-6 weeks of monitoring.

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

The majority of mice used in this project will not suffer any adverse effects, as they will be used in Protocol 1 for breeding before *in vitro* experiments. Mice used in other protocols do not all suffer adverse events, as a large amount will be used as controls, or will be culled early in the process before illness develops.

However, some mice in protocols 2-6 will suffer adverse events. Expected effects are (but are not limited to):

Protocol 2 - inflammation at the site of injection with adjuvant

Protocol 3 - inflammation at the site of injection with adjuvant; development of flaccid tail; paralysis in hind legs; weight loss; hunching; lack of grooming.

Protocol 4 - adverse effects develop from the burden of the tumour as it grows. These can include weight loss; hunching; lack of grooming; reduction in mobility.

Protocols 5 and 6 - inflammation of the intestines results in weight loss; diarrhoea; bleeding from the rectum; hunching; lack of grooming; reduction in mobility.

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)?

Sub-threshold - all mice used for breeding (as no GA mice are expected to show a phenotype at breeding stage); all control mice - expected 50% of total mice

Mild - all mice in protocols 2-6 which are culled before signs of illness develop; all mice in protocols 2-5 which experience no weight loss or signs of illness - expected 20% of total mice

Moderate - all mice in protocols 2-6 which develop signs of illness - expected 30% of total mice.

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?

Our work is deeply rooted in understanding the physiology of human disease, and as such all initial work is performed on human samples (peripheral blood T cells and neutrophils, tissue blocks from pathology departments, and fresh tissue from biopsies). However, not all work can be performed using human cells. In particular, examination of inflammation of the central nervous system is very difficult and highly invasive for patients. Our work also involves understanding very early events in the development of pathology, when patients are unlikely to know they are ill and so will not be attending clinics.

The majority of our work is performing *in vitro* experiments on cells isolated from mice culled by schedule 1 methods. This allows us to understand mechanisms of disease, and generate hypotheses. However, these do not answer questions about the complex interplay of multiple immune cell subsets which form a large part of our work. Therefore, *in vivo* models of inflammatory disease are also necessary.

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

1. Cell lines

2. *in silico* analysis of previously published datasets

3. use of human peripheral blood cells

Why were they not suitable?

1. There are good T cell lines, such as Jurkat cells, which we make use of in the lab. However, neutrophils are terminally differentiated and not genetically tractable, and there is no physiologically-relevant neutrophil cell line.

2. We are developing expertise in the lab at analysing published datasets of single cell RNA sequencing, bulk RNA sequencing, and proteomic experiments performed on human and mouse T cells and neutrophils. These are proving useful to support and develop our studies. However, they cannot replace experiments in our multiple lines of GA animals, which are relatively rare lines in which few experiments are performed.

3. A large number of experiments in our lab use cells isolated from human peripheral blood, and these are the foundation of our work. However, it is difficult to model cell migration through tissues or the complex interplay of multiple immune cell subsets *in vitro*. In addition, it is not easy to keep cultures alive and in a physiological state for months in order to examine the impact of neutrophils on memory or exhausted T cells.

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 are informed by the numbers of mice used in the previous project licence. However, since that licence significant funding has been won by the research group and so staff numbers have increased, meaning more experiments will be performed.

Numbers of mice used in breeding (protocol 1) are informed by the typical numbers used in current strain breeding. A large number of mice are used in this protocol owing to the multiple transgenic lines in use. In experimental protocols (2-6), the numbers are estimated from group sizes and experimental plans in our funding proposals.

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

We continually examine our procedures and protocols to optimise methods such that fewer mice need to be used overall. We are also developing totally new protocols in the lab to analyse published data collected by other teams, so that we can bypass experimentation entirely in some areas.

We aim to set experimental group sizes so that data are usable and reproducible, and robustly answer the scientific question being asked, with no wasted mice. We aim to publish all data in accordance with ARRIVE guidelines. We have a robust system of pilot experiments then power calculations in order to set final groups sizes. Control groups are when possible shared between studies and internal controls used (for example, using contralateral lymph nodes as control for tumour draining lymph nodes, rather than other untreated mice).

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

Tissues are shared between experiments, such that one wildtype uninfected mouse can serve as a control for DSS colitis (small intestine), EAE (brain) and inoculation (lymph node) by different staff members.This reduces the number of mice used significantly.

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.

Experimental autoimmune encephalomyelitis: this is currently the best animal model we have of Multiple Sclerosis, and multiple drug treatments given to patients were developed using this model. We have refined the usual EAE protocol over the past five years to ensure the suffering is reduced to the minimum possible. For example, we have halved the amount of pertussis toxin used, so that the severity of disease is lowered and onset is slower (such that overall suffering is reduced).

In order to study how immune cells move into and are activated in the central nervous system, it is necessary to allow the model to develop to a point where the animals experience suffering. However, we endeavour to end experiments as early as possible - the majority of our experiments are ended within 14 days. Mice are monitored extremely closely during these experiments and refinements such as softened food placed in the cage are used to lessen their suffering. Water bottles with long spouts so that the mice can easily access water will also be used. We have switched to using commercial kits of pre-prepared myelin in adjuvant. This gives completely reproducible disease where 100% of mice develop the same signs of disease on the same day. As a result, fewer mice have to be used and the variability is lower. This means we can also control the amount of antigen given to the mice and can lower it so that the disease severity is reduced.

DSS colitis: this model is used to understand more about how immune cells drive development of inflammatory bowel disease in patients. We perform careful dose studies in each genotype of mice used as the disease can vary significantly between strains. Pilot studies are used to assess disease course. We very carefully monitor mice to ensure suffering is kept to a minimum.

Development of solid tumours: we use these models to understand how T cells move into tumours in patients, how they are triggered to develop and to become exhausted, and how they produce different cytokines in different situations. Suffering of the mice is limited by our very careful monitoring of tumour development, including frequent measuring of the tumour size as well as weight loss of the mouse. We end the experiments as soon as we can, which is often early in disease course as we are interested in how early T cell responses are generated in lymph nodes.

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

It is difficult to perform T cell studies or analyse lymph nodes in less sentient animals as the cells are frequently not present or are too different to human T cells to be useful as a model. This is true, for example, of zebrafish larvae, Drosophila and C.elegans. The reagents and protocols we need to use to answer our scientific questions are most established in mice.

As we study the development of T cell responses over a time course of days, it is not possible to use terminally anaesthetised mice.

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

We continuously review our processes and protocols, in discussions within the lab and with outside experts such as animal technicians, veterinary staff and collaborators in other institutions. This enables us to refine our experimental processes in line with best practice.

Refinements have been put in place for the EAE model after extensive discussion. We are now aiming to replace the use of Complete Freund's adjuvant in protocol 2, with an adjuvant which induces less inflammation at the injection site. Pilot studies will be performed, informed by published studies, to determine if we can use other adjuvants in this protocol and still see robust T cell activation.

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

We follow the NC3Rs guidance and aim to publish all our work in accordance with the ARRIVE guidelines.

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

The establishment runs frequent training days on the 3Rs, and is developing a community of researchers who meet to discuss these issues. In addition, best practice is informed by discussion with collaborators and attendance at conferences in which these topics are discussed.

I am signed up to the NC3Rs newsletter, which discusses the 3Rs in depth.