Home Office

NON-TECHNICAL SUMMARY

Genes and environment at the interface of inflammation, immunity and metabolism

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Inflammatory bowel disease, Immunity, Metabolism, Cancer

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?

We aim to elucidate how risk genes of human inflammatory diseases operate, and how they contribute to 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?

Enormous efforts have been made to identify inherited risk factors of disease. The inflammatory bowel diseases Crohn's disease and ulcerative colitis have been amongst the most successfully studied in this regard, with now >250 spots in the genome identified that increase risk for disease. What is often overlooked is that only a handful of those identified genomic loci are biologically well understood, meaning that it is known how they mechanistically contribute to disease. For some of these risk loci, not even their basic function is known. Our group has a long track record in deciphering major mechanisms of Crohn's disease and ulcerative colitis by studying genetic risk factors. As examples, by studying specific genes, we have defined how two cell biological processes, called 'endoplasmic reticulum stress' and 'autophagy' contribute to the disease process. In this study we will investigate how further IBD-associated genes, which we have identified as intricately linked to cell metabolism, contribute to disease pathogenesis.

Variants of a number of risk genes predispose for Crohn's disease and ulcerative colitis, two inflammatory bowel diseases; for primary sclerosing cholangitis, a chronic inflammatory liver disease; Still's disease, a childhood fever syndrome with debilitating arthritis; and leprosy, a chronic bacterial infection of the skin. They hence predispose for major human diseases spanning 'autoinflammation' and 'autoimmunity', meaning diseases where the immune system appears to attack the body and organ. Ulcerative colitis and primary sclerosing cholangitis are also the diseases with the highest risk of developing inflammation-associated cancer.

Our studies will identify key mechanisms of disease, a prerequisite to develop effective treatments. All of these conditions remain poorly understood, and for some of them, such as primary sclerosing cholangitis, there is not even a single approved medical treatment available. Insight gained from our studies are very likely to have ramifications way beyond the diseases the risk genes are associated with, given how fundamental for cellular energy metabolism their function is.

Studies such as ours are key to fill 'risk coordinates in the genome' with 'functional life' - a prerequisite to allow for the genomic revolution in medicine, an area where the UK is world-leading, and one that is the very focus of the government's life sciences strategy.

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

We will generate new insight into the major biological processes underlying inflammatory diseases. We will publish these insights in scientific journals, and present them at conferences.

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

Projects such as ours are the very basis for future therapeutics. Patients suffering from Crohn's disease, ulcerative colitis, primary sclerosing cholangitis, Still's disease and leprosy, as well those suffering from cancer, will ultimately benefit from these outputs. Mechanistic insight into major disease processes has been the basis of breakthrough treatments for these diseases - examples are new dugs that target inflammatory mediators called Interleukin-12 and Interleukin-23. We expect very similar outcomes and hence benefits from our research here, too.

More immediate beneficiaries will be the scientific community, both in academia and in industry. This research will advance insight into major disease processes. Given the specific focus, there is also a high likelihood that insight gained through this programme will be important well beyond the inflammatory diseases the genetic association is with. Specifically, we think that outputs from this programme will be important for researchers working to understand cancer and metabolic diseases.

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

We extensively collaborate and share new results early-on. Large data-sets will be deposited at databases for other scientists to use and exploit. We present our work at conferences prior to publication. We often report on unsuccessful approaches and non-significant data, too, since these are often as valuable as successful experiments and their results. We typically publish our work in major journals, which maximises the visibility of our work.

As we have in the past, we will seek patent protection where and when appropriate. This enables the development of new therapeutics, exploiting our discoveries.

Species and numbers of animals expected to be used

- Mice: 20,000

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 mouse is the most appropriate animal to investigate human genetic risk factors of disease. In the mouse, genes can be altered, which allows us to alter those that are involved in human disease. The immune system and metabolism are very similar between mice and man, and our understanding of mouse physiology is very deep. There are also many experimental tools, such as genetic models, recombinant cytokines (these are molecules that signal between cells) and antibodies, available for mice.

We investigate risk genes for human autoimmune/autoinflammatory diseases. The risk genes we are interested predispose for the inflammatory bowel diseases Crohn's disease and ulcerative colitis; a childhood arthritis with recurring fever that is called Still's disease; the liver disease primary sclerosing cholangitis; and the chronic bacterial infection leprosy.

All of these diseases arise from a complex environment - gene interactions. They involve numerous different cell types and organs. An example is the gastrointestinal tract, which contains many different cell types in a particular architecture within the organs. These include the intestinal epithelium, which is the inner barrier of the body, and which itself is composed of several different cell types; multiple types of immune cells, such as macrophages, dendritic cells, T and B lymphocytes; fibroblasts, endothelial cells (cells that cover the inner lining of vessels), neurons, etc. They are all involved in the disease process. The intestinal tract also contains the intestinal microbiota, an enormous accumulation of diverse bacteria, viruses and fungi that actually outnumber the cells of the human (or mouse) body. Over the last decade it has emerged that the microbiota has a huge impact on many diseases, including a particular large one on those we are studying.

Unfortunately there are no cell culture systems in a dish that come any close to modelling this vast complexity both on the host side, and on the microbial side.

Regarding life stage, we will generally study adolescent and adult mice.

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

We will generate, breed and maintain genetically-altered mice in this project.

Almost all of the mice we generate will carry genetic alterations. These genetic alterations are typically not harmful or even noticeable, and mice breed and develop normally.

Only few mice will carry mutations that lead to spontaneous phenotypes, specifically mutations that result in tumour development.

Small tissue samples will be taken to determine the genotype of mice. This is typically done at an early age (3-4 weeks).

Most genetically altered mice will not have *any* further interventions performed on them. They will serve as donors of tissues or cells, commonly harvested at an age of 8-16 weeks. Cells and tissues will be obtained from dead animals or during terminal anaesthesia. Most of our investigations will be reliant on primary cells and tissues obtained from these mice. Cells and tissue will then be studied in the laboratory in primary cell cultures in a dish, or analysed for gene or protein expression, or for metabolites.

Only a minority of animals will undergo further procedures:

In some mice, their metabolism will be characterised. This may require housing them for 2-5 days in special cages that monitor energy consumption. Occasionally, single mice will be housed in individual cages. Few mice will be fasted for up to 18 hours, and small amounts of blood will be taken from the tail vein at several time-points after metabolic 'fuel' (e.g. glucose) has been administered.

Some mice will be housed in special isolators that prevent colonisation with microbes. Such 'germ-free' mice generally develop normally. They may then be colonised with specific microbes, which are typically administrated through the mouth via oral gavage.

Mice may also be injected with agents, administered into the tummy (i.e. into the 'peritoneum') or underneath the skin. Or agents may be administered through the mouth via drinking water, food, or per gavage; or via the nasal route. Agents may include substances such as drugs, metabolites, immune-stimulating reagents. They may also include microbes such as viruses or bacteria. Colonisation experiments may last several weeks, infection experiments typically 1 to 4 weeks, depending on the agent.

On few mice we will conduct imaging studies, such as computer tomography. This may require administration of imaging agents.

Depending on the route of administration and type of agent, we may apply a local or general anaesthetic prior to the agent.

Some animals will be injected with tumour cells to study tumour growth and how this can be treated. These experiments typically last 3 to 4 weeks.

Very rarely we will induce overt disease in mice by administering agents that cause colitis (inflammation of the gastrointestinal tract) or arthritis (inflammation of the joints). The duration of these models varies from few days to few weeks.

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

The majority of mice will not experience more than mild and transient discomfort. This is due to when a tiny piece of tissue is taken for genotyping.

Most of the genetic alterations we will study do not cause spontaneous harmful phenotypes.

Mice prone to develop intestinal tumours may stop gaining weight or may lose weight. Tumours typically develop slowly over many (12-20) weeks.

Administration of immune-stimulating reagents, infectious pathogens or colitis/arthritis-inducing agents can cause abnormal behaviour, reduced food intake, pain and weight loss. The duration of clinical symptoms varies between models, typically lasting from few days to few weeks.

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 majority of mice will fall under the 'sub-threshold' or 'mild' severity banding.

A minority of animals will develop 'moderate' symptoms. We will not pursue experiments that would fall under the 'severe' banding.

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 research programme uses a wide range of approaches to elucidate disease mechanisms. These range from the atomic/molecular (e.g. structural biology), protein-based (e.g. enzymology), to cell culture in a dish. The latter includes 'permanent' cell lines (typically meaning cancer cell lines that can indefinitely be grown in a dish), genome-edited human pluripotent stem cells (iPSCs), and the *ex vivo* experimentation with 'primary' cells and tissues that are isolated from mice.

For every question we ask, we always use the simplest model system that is available.

We only use animals (mice) when there are no other suitable models available to answer specific questions that arise from our overall research programme. In fact, research involving animals are only a small part of our overall research programme into mechanisms of human disease. We use them judiciously and carefully when no alternatives exist to answer an important question.

The very nature of the biochemical mechanisms we investigate limits the use of *permanent* in vitro cell cultures. This is why we have to rely in these instances on 'primary' cells (meaning, these are taken out of a living organism - e.g. from humans or mice). It is important to appreciate that the proteins we study are 'polymorphic' in the human population, meaning the protein sequence differs at specific amino acids between individuals. To demonstrate what the consequences of this variation is, we have genome-edited the corresponding parts of the mouse genome. This allows us to study them on an identical genetic background.

Many aspects of human disease processes cannot be modelled *in vitro*. This is very apparent for intestinal inflammation, which involves multiple different cell types (various T and B lymphocytes, macrophage and dendritic cell types, innate lymphoid cells, natural killer cells - these are all immune cells; fibroblasts, epithelial cells and their subtypes, endothelial cells, neuronal cells, and many others) and involves complex interactions with the intestinal microbiota, the enormous amount of diverse bacteria (and other forms of life) in our intestine that actually outnumbers the actual human cells. Neither the host side, nor the microbial side can be modelled *in vitro* to any meaningful extent. Whilst a microbiota involvement might be most obvious for intestinal inflammation, it has become very clear that it plays a similarly huge role for inflammation at other sites, for the organisms' metabolism, and may even determine the effectiveness of differnt types of cancer treatment.

The extremely high conservation between mouse and man of the genes and pathways we study, and the close similarity of the mouse and human immune system and metabolism in general render the

mouse the most appropriate model system.

Finally, the biochemical mechanisms we have discovered and that underpin our research programme have huge ramifications for organ and inter-organ physiology. Again, this could not be studied in a more reductionistic system than an in vivo model.

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

Mouse experimentation forms only a small part of our overall research programme into human disease mechanisms. Our mouse work is embedded in a much larger research programme that uses a wide variety of technology.

We are using structural biological methods, protein chemistry, enzymology, metabolomics, and various other approaches to gain fundamental insight into mechanisms of disease. These are methods that let us study the proteins themselves that we are interested in. They let us often study even single atoms and help us understand how these proteins work. These studies form a central and large part of our overarching research programme.

At a further level of complexity, we want to understand how these proteins operate within a cell. For this we use cell culture in a dish. We employ permanent cell culture of various (cancer) cell lines (these have names such as MODE-K, Caco2, HEK293, THP1, B16, etc), as well as complex 'organoid' cell cultures (these are 3D cell cultures that can resemble the basic architecture of the cell type that is characteristic of a specific organ), including those derived from human induced pluripotent stem cells (iPSCs). We have experience in using gene-editing techniques to edit the genome of human iPSC to toggle the put the human gene variants into their genomes. Such 'toggling' is important to study what consequences a specific human variation has.

We also perform studies with cells isolated from peripheral blood from healthy individuals. The latter allows us to identify individuals who are homozygous for either variant of the polymorphic genes we study.

Experimentation involving primary cells from genetically-altered mice, and *in vivo* experimentation in mice form only a small part of our overall research programme and are used when absolutely necessary.

A large part of our work involves the activity of the immune system. Since the molecules that present antigens are highly polymorphic (this means they are different between every human) makes many studies of antigen-specific immunity impossible in cells from cohorts of humans - even when disregarding that the site of primary immune-stimulation is in tissue which would generally not be accessible for experimentation in humans.

We have extensive experience in all of these methods and use them on a regular basis. We constantly re-assess what the most appropriate experimental approach is.

Why were they not suitable?

As already alluded to above, many aspects of human disease processes cannot be modelled *in vitro*. This relates to the variety of cell types involved; the environmental signals/cues; the microbiota and many others. Many relevant cell types are not accessible at all in e.g. peripheral blood from humans.

An example of the challenges we are facing is the intestine. While 'organoids' are useful model systems, these are clearly not mini organs. For example, intestinal organoids show a 3D structure reminiscent of the intestinal epithelium (the cell type that forms the single-layer cellular interface with what is inside the lumen of the intestine, meaning the enormous amount of bacteria alongside the food that is being ingested and digested), but they do not contain the myriad of immune cells, muscle cells, vessels, fibroblasts and neurons that form our intestine. They clearly also do not contain the unique ecosystem of bacteria, viruses and fungi that is present inside our intestine.

A particular challenge in our case is that the proteins we are studying are polymorphic, hence we would need to 'pick' healthy individuals for homozygosity of the risk and non-risk genotypes. We have indeed conducted select experiments with the support of individuals, but for reasons that are obvious these individuals could not possibly supply our research programme with a continuous supply of primary cells. Since a large part of the project involves studies of the immune system and the antigen presenting molecules and the receptor that recognise these antigens differ from person to person (they are highly 'polymorphic'), it would be impossible to find individuals that have either of the variants of interest, but are identical for the immune receptor.

Finally, the very nature of the biochemical mechanisms we investigate limits the use of *permanent* in vitro cell cultures, albeit we use them extensively whenever this is possible. These in vitro models also have obvious major advantages for mechanistic studies, due to the simplicity of their pharmacological or genetic perturbation.

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?

By keeping our experimental conditions well controlled we are able to perform highly reproducible and statistically meaningful experiments using the minimal number of animals. The experimental approaches described herein have been vigorously evaluated over the past two decades, thereby providing us with a wealth of data that help us optimise experimental design and achieve robust data from the minimal number of mice used. We have extensive experience in experimental design and statistics. Post-doctoral scientists in our group have been on experimental design courses to help understand sources of bias and variation and how best to reduce them. We also have access to external advice on statistics, if required, to help guide experimental design. Where possible in our design we blind people to genotype/treatment groups with different people doing infections or analysis.

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

For our various experimental approaches, be it *in vitro* experimentation on primary cells, or *in vivo* experimentation in particular models, we have extensive data from various contexts. These insights are invaluable for designing robust experiments with the minimal number of mice required. We have used the NC3R's experimental design assistant for work we have done on previous studies, and will continue to use it in future studies. The PREPARE and ARRIVE guidelines have been consulted for formulation of this project, and these will be followed to ensure continued communication between animal facility and our team, and reporting data from our experiments. We also collaborate extensively, seeking input and advice from colleagues who are experts in particular models.

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

When required and as part of this programme, we will use pilot studies to inform us on the control experimental conditions we need to use. We have extensive experience in the *in vivo* inflammation, infection and tumour models in this project, and thereby access to a wealth of data that make predictions of effect sizes and optimisation of experimental strategies easier. In an iterative way, we are thereby able to continually improve and optimise our strategies.

Please also note that these experiments involving genetically-altered mice is embedded in a much larger research programme that uses a wide range of different techniques to elucidate mechanisms of disease. These range from 'atomic resolution', using structural biology approaches, to enzymology, assays on recombinant proteins expressed in various in vitro expression systems, to a wide range of cell culture work, also involving human induced pluripotent stem cells that are being genome-edited. It is a typical feature of our programme that results from e.g. structural or enzymological experiments raise hypotheses that trigger experiments in cell culture or in cells from genetically-altered mice; and *vice versa*. This integrated, comprehensive and iterative approach allows for very judicious use of animal experimentation, with the constant aim to reduce the numbers of mice needed.

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 vast majority of genetically altered mice generated on this license will not undergo any procedures apart from genotyping, hence will not experience pain, suffering, distress or harm. These mice, once dead or terminally anaesthesised, will serve as donors of primary cells and tissues. The genetic modifications we study do typically not cause spontaneous phenotypes.

A small minority of mice will undergo procedures. These include adminstration of reagents or cells; infections with pathogens; induction of inflammation, such as colitis and arthritis; and induction of tumours. We believe that the similarities in the mouse and human genomes are such that we can infer between the two, and we have closer links than ever before with patients suffering from these conditions. Over the years we have gained tremendous experience with our models and, through careful observation, we are able to minimise the potential suffering of the animals. We have been able to identify key clinical signs that indicate illness and consequently such animals can be quickly and humanely killed.

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

As noted above, most of the mice generated on this license will be used as organ, tissue or cell donors when they are dead, or after terminal anaesthesia.

It is not possible to model all aspects of tissue inflammation, such as in arthritis or colitis, of organ and systemic infection, tumour formation, and tumour immune responses outside of the whole animal. The mouse is a very good model system as it closely resembles human physiology. More sentient systems, such as Drosophila or fish, have very different immune systems and lack defining parts of the human (and mouse) immune system.

Complex interaction of various host cells and -metabolites, and with the environmental cues and the microbiota are critical in the disease processes we study. There are no 'more reductionistic' model systems available that would reproduce the processes in human disease to a meaningful extent. We recognise that our mouse model systems have limitations and cannot reproduce all the conditions associated with human disease. However, mice have many similarities to humans, including in terms of their immune system and metabolism.

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

Mice are monitored throughout all interventional experiments and we collect daily scores composed of a set of physical signs of illness such as piloerection (raised hair as a sign of pain or discomfort), hunched walk and mobility along with weight loss. The cut-off for these physical signs lies within the guidelines for mild, and in a few contexts, moderate severity, i.e. loss of pre-set percentage body weight being our main indicator, along with mobility (ability to feed and water). The scoring for piloerection etc. are also used as secondary indicators. My team are experienced in animal models and are trained to the high standards that I expect. The technicians that work in our holding facility and do the majority of the animal husbandry will also be trained by my team and will communicate abnormal behaviours in the mice early. At our establishment we have dedicated Named Animal Care and Welfare Officer who are impartial and can give advice / make decisions on animals that lie outside of the normal adverse effects expected for the infections outlined within this project.

Potential refinements include increased monitoring if test animals show earlier clinical signs or weight loss. We will give wet mash food to animals that lose more weight quickly. Floor food will be given to animals that are to be infected to limit weight loss from the start of the infection.

Any evidence in pups not developing or litter losses will be discussed with NVS and appropriate actions taken.

All animals will be given environmental enrichment and be socially housed to encourage natural behaviours and reduce stress. Males will be monitored for increased aggression and additional enrichment added to combat or appropriate splitting of fighting animals if needed.

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

We will use guidance from the NC3Rs website and the Laboratory Animal Science Association (LASA) to ensure experiments are conducted appropriately. In particular we will follow the 'Guiding principles on good practice for Animal Welfare and Ethical Review Bodies'.

We will follow the PREPARE guidelines for planning experiments and will follow the ARRIVE guidelines reporting of results.

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

I will keep informed on advances through the NC3R’s website, Norecopa website, our establishment website and newsletter. We will discuss any advances with the relevant people at our establishment and implement them accordingly.