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NON-TECHNICAL SUMMARY

Environmental influences on immunity and tissue biology

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

mucosal immune system, intestinal stem cells, environmental signals, cancer, infection

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

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 study how environmental triggers transmitted via the aryl hydrocarbon receptor affect immunity and tissue biology

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?

While it is known that genetic traits contribute substantially to how the immune system works to protect against pathogens and tissue damage, it is equally clear that environmental factors strongly impact such responses. Our studies have identified a ligand dependent transcription factor, the aryl hydrocarbon receptor (AHR) as a molecular entry point for environmental factors that can be man-made pollutants (xenobiotics) or physiological ligands derived from the diet or the microbiota. Stimulation of AHR with physiological ligands has beneficial effects for maintaining the integrity of the intestinal barrier, acting on different immune cell types as well as tissue cells such as epithelial and endothelial cells. We are working on the underlying mechanisms to understand why physiological ligands are beneficial whereas xenobiotics have detrimental effects on health.

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

At the end of the project we anticipate to have a greater understanding of how environmental factors transmitted via the AHR influence the immune system and tissues in steady state as well as after challenges such as infections, tissue damage or tumorigenesis. We will disseminate our results in conferences and publications as well as in public engagement events.

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

Since many of the natural AHR ligands are derived from the diet, it is possible that dietary supplementation can be used for preventative purposes or for alleviation of inflammatory reactions particularly in the gut. In the long term this might be applicable to humans with intestinal disorders or the genetic predisposition to develop such disorders.

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

We are collaborating with toxicologists who focus on the detrimental effects of xenobiotic AHR ligands to understand the underlying reasons for the different outcomes following exposures to xenobiotics compared with natural AHR ligands. We also have collaborations with groups that work on infection of the lung as AHR seems to play a role in tissue repair following infection. We have written a comprehensive review on the role of AHR in the intestine for Nature Reviews in Gastroenterology and Hepatology and I am frequently asked to give seminars or conference presentations on our research.

Species and numbers of animals expected to be used

- Mice: 30000

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.

We are using mice genetically modified to have deficiencies in the AHR pathway in different cell types using Cre lox systems in order to evaluate where AHR is needed to protect against infection, inflammation and tumorigenesis. In most of our mice AHR is deleted or altered genetically so that the mice express these genotypes from birth. However, we also will use tamoxifen inducible Cre strains to be able to induce deletion or alteration at certain life stages. This is particularly important for assessment of AHR in endothelial cells as the existing literature suggests that AHR shapes vessel maturation early in development. For this reason we will need to use some neonates and juvenile mice to evaluate the consequences of AHR deletion early in their life, compared with deletion in adult stage. We anticipate that AHR roles early in life may be recapitulated in adult mice upon injury which requires restoration of vessels.

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

In a typical scenario animals might be subjected to administration of substances to induce gene deletion and/or activate or inhibit AHR. They might then be infected with a pathogen . Following such treatment the mice might be subjected to imaging under anaesthesia, they might have blood taken for analysis (and will finally be killed typically about 4 weeks after Step 1, which may involve perfusion or exsanguination under terminal anaesthesia. A minority of animals (<10%) might in addition experience irradiation followed by bone marrow reconstitution or transfer of immune cells as well as treatment with antibiotics. Protocol 5 (Induction of colon tumorigenesis) will follow mice over 12-16 weeks as induction of tumorigenesis, especially in wildtype mice is a slow process.

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

Animals may experience pain, weight loss and tumours for a short period of time

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)?

Immunocompromised mice and those lacking AHR might be more susceptible to the procedures and reach the moderate severity limit of the protocols.

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?

While it is possible to generate information regarding regulation of some aspects of the immune response in cell culture it is not possible to mimic the response to infection or intestinal disease in vitro, since immune cells are highly connected with tissue components and crosstalk between non-haematopoetic, eg epithelial cells and immune cells is an essential feature of inflammatory responses.

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

We will where possible, collect and generate as much data as possible using in vitro cellular immunology assay systems. For instance, intestinal homeostasis and its perturbation in genetically modified mice may be investigated using organoid cultures of intestinal epithelial cells.

Why were they not suitable?

In vitro systems, while useful, do not fully replicate the complexity of immune interactions or disease pathogenesis in vivo and it is essential to use appropriate and robust animal models to dissect these processes. Furthermore to develop therapeutic approaches with potential to alleviate human disease it is necessary to establish parameters influencing efficacy in an animal model.

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?

For the design of most of the quantitative experiments, sample sizes will be set using power analysis, generally using a significance level of 5%, a power of 80% and at least practicable difference between groups of 20%. Otherwise we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own or from the literature). Wherever possible, factorial design will be employed to maximise information using the minimum mouse number. Statistics expertise will be sought within the institute, and active statistical discussion with bioinformaticians. Pilot experiments will use between 5-8 mice per group, which should be sufficient if a significant result is obtained. Experiment will be repeated to obtain further significance if there are only small differences; in this case it may have to be repeated with larger numbers of mice and/or modifications.

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

Longitudinal measurements, in particular non-invasive ones, such as weight loss, clinical scores and non-invasive imaging, allow gaining a wealth of information on disease course over time with minimum number of mice used. Cryopreservation of gametes, embryos, tissues and cells is routine at the establishment and will ensure that the minimum number of mice is bred, and measures are in place to maximise efficiency of breeding schemes with minimum surplus. Reporting will be based on ARRIVE guidelines. Imaging technology will allow following a cohort of mice over time rather than setting up several experimental groups to allow kinetic analysis of eg the consequences of an infectious stimulus. Furthermore, colonoscopy with an endoscope will be a monitoring method for the colitis and tumorigenesis protocols to allow us to detect inflammation and tumorigenesis earlier and without the use of large cohorts of mice. We will remain alert to any advances, which will enable the replacement of animals.

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

The efficiency of animal usage is maximised in consultation with animal technicians, by careful control of breeding to meet research needs with respect to numbers, phenotypic uniformity and health. This has been greatly facilitated by a mouse database in which every breeding pair and every mouse born are recorded and through which we can readily monitor the numbers of mice we hold. Where possible lines will be maintained in a homozygous state, thereby obviating offspring with undesirable genotypes. Littermates genotyped as heterozygous or wild type from the breeding protocol will be used as appropriate age and gender matched controls. This allows optimal use of mouse numbers generated and is best scientific practice for the study of genetic alterations.

For experiments on tissue inflammation or cancerogenesis in response to infection we will wherever possible make use of bioluminescence imaging to follow a cohort of mice over time, which will substantially reduce the number of mice involved. Lastly, this programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. This highly integrative approach will maximise the information obtained from the minimum resource.

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 models of infection, tissue inflammation and tumorigenesis are the best established models in the field with high relevance to human disease states. They are generally non-invasive and are likely to

yield significant results without causing lasting suffering and pain.

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

The mouse model allows for control over host genetic factors, which is crucial to studying the pathogenesis of disease. With these models we can investigate how the immune system recognizes and responds to infection, how tissue damage is repaired and how tumours arise which can lead to new avenues of treatment and prevention.

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

We will work closely with the veterinary staff to ensure that we are always refining our protocols to minimize harms for the animals we work with.

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

We will stay up to date with the best practice guidelines developed by the National Centre for the Replacement, Refinement, & Reduction of Animals in Research, and the scientific literature for estimation of sample sizes based on power calculations.

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

We keep up with the latest developments in the field by reading the relevant literature and we have ongoing discussions of 3R measures in the institute which ensures we are always up to date.