Home Office

NON-TECHNICAL SUMMARY

What's the aim of this project?

We want to understand how lymphocytes interact with other cells types to promote immunity, resilience and repair at the organismal level. To achieve this we will combine physiological, cellular, molecular and computational approaches across the life course.

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?

The immune system is now understood to be a collection of distinctive cell types that mediate immune effector functions, such as antibody production or cellular cytotoxicity, to promote immunity. However, the molecular basis for this, including the genes that promote the durability of immunity to infection and limit the effector functions of immune cells to avoid harmful reactions against self and promote resilience remains to be understood. The molecular and cellular basis of how immune cells promote the healing and repair process is essential to understand how these mechanisms operate optimally and deteriorate as organisms age and will be fundamental to treating the common human diseases of the 21st century.

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

We will continue to publish our findings in peer-reviewed journals making use of open access, preprints

strains a small number of female mice (<500) will receive embryos via surgical or non-surgical procedures (Protocol 2). A very small number of male mice (<20) will be vasectomised to produce pseudo-pregnant embryo recipients (Protocol 3), these males will be kept until twelve months of age.

We will breed and maintain genetically altered (GA) mouse strains for experimentation. The maintenance until a maximum of 15 months of age will cover the mice used in this study under Protocol 4 where no or rare (<10%) mild effects are anticipated (< 84000 procedures). The majority of mice of both sexes will be studied either after killing by a humane method to address questions on pseudxpe[illegible] to address questions on

While we recognise limitations of mouse models and the need, where possible, to verify findings in other systems and species, we emphasise the mouse has proven overwhelmingly successful for the discovery of new cell types and the molecular genetic mechanisms underpinning their function. It has yielded essential basic knowledge of the immune system now being applied to improve the health of the humans and economically-important mammals.

Furthermore, it is extremely difficult to study lymphocytes in the bone marrow or lymphoid tissues of humans, because it is very difficult to obtain these from healthy donors.

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

We have considered using cell culture methods, including organoids and do use these when appropriate. For example, we are using an *in vitro* model to investigate molecular pathways involved in germinal centre formation and plasma cell differentiation to complement the *in vivo* studies.

Why were they not suitable?

Features such as the distribution of lymphoid organs throughout the body and the intrinsic properties of lymphocyte recirculation cannot be recapitulated in tissue culture or organoids making investigations in the whole animal context essential.

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 have estimated the number of animals we will use based on our previous studies using these protocols. The numbers of mice required for the generation and rederivations of genetically altered mice are based on extensive experience of staff who regularly perform these protocols.

The use of colony management software and knowledge of the breeding performance of individual strains has enabled us to predict the numbers of mice of the correct genotype that we will produce from breeding, and the numbers of aged mice that we will need.

The numbers of mice required for experimental groups are based on power calculations, appreciation of variability and a knowledge of biologically meaningful effect sizes. We have factored in the need for experiments to be replicated independently and for greater variation in the responses of aged mice.

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

We reduce the numbers of animals used by paying careful attention to the design and planned analysis of the results including consultation when needed with a biological statistician in the Bioinformatics department and we use the NC3R's Experimental Design Assistant to ensure we are considering all relevant aspects of design. We perform our experiments in carefully controlled animal facilities that reduce biological and environmental variation; the use of optimised experimental procedures, including the use of genetically identical strains, blinding and randomisation to reduce technical variation; the multiparameter analysis of individual mice; the adaptation of new technologies such as the use of ribonucleoproteins to create knockouts or other genetic modifications; improving the sensitivity of techniques to enable measurement to be made on small cell numbers isolated from a single animal. For some experiments we are using in vitro methods to promote cell differentiation to defined states under controlled conditions, thus enabling access to cell numbers that would be unfeasible to obtain from mouse tissues.

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

Where possible we will share mouse strains rather than generate new strains by transgenesis. Our mouse facility has an extremely efficient pipeline of tissue biopsies and rapid genotyping by a commercial provider, that provides timely results to enable efficient breeding.

Selective deletion of individual genes in specific tissues will be achieved by the use of Cre-Lox recombination to delete the gene of interest in defined cells. We employ rigorous quality control to ensure the fidelity of these systems. Pilot studies ensure minimum use of mice before decision points are reached in experimental design. emMetʃ

lymphocytes differ significantly from humans and mice making them interesting but unsuitable for our project.

The mouse is the species of choice because of the extensively validated tools and resources available for quantitative immunological phenotyping and mechanistic studies across the life-course. Studies of ageing in the mouse have provided key insights into human immune systems but require the long-term care of mice with which we have much experience. The availability of inbred stains and well annotated genomes is important for our studies as they develop into the investigation of molecular mechanisms *ex vivo* and *in vitro*. The requires careful and attentive monitoring of breeding programmes and quality control.

Early-stage mouse embryos are unsuited to these studies as the adaptive immune system and immunological memory is a feature of adult animals and we propose to study these features in adulthood and ageing animals.

Terminal anaesthesia is inappropriate for long-term experiments that require systemic immune responses, but may be used to prepare tissues for further study.