Home Office

NON-TECHNICAL SUMMARY

Lymphocyte development and ageing

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

lymphocyte development, ageing, antibodies, gene regulation, signalling mechanisms

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

The aim of this project is to understand gene expression and protein interactions that regulate development of B lymphocytes and antibody production, and how these change in ageing and 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?

B lymphocytes generated in the bone marrow produce the enormous antibody repertoire that we need to fight millions of different infections we may encounter. We do not fully understand many of the gene regulation and signalling mechanisms required to produce sufficient numbers of B cells with sufficient quality of antibodies throughout our normal lifespan. Undertaking this work will help to reveal how B cells progress through multiple stages of development, and how they convert a few hundred antibody genes into millions of different antibodies. This will help us to understand why some people fight infections well, while others do not, and may identify new therapeutic strategies that could be used. More specifically, older people are less able to fight infection and to benefit from vaccination. This is partly because older people make fewer B cells in the bone marrow, and these B cells make fewer different antibodies. If we can understand the major pathways that are defective in ageing, we can design strategies to reduce these defects and boost the ageing immune system. This will make a vital contribution to improving healthspan (how many years we stay healthy) in our increasingly long-lived population. Unfortunately healthspan is not currently keeping pace with lifespan, resulting in an ageing population that is living longer with multiple health conditions that reduce quality of life. Finally these studies will also contribute to our understanding of immunodeficiency diseases caused by poor antibody responses, and to our understanding of B cell leukaemias that initiate in bone marrow B cells.

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

Using state of the art genome-wide sequencing and computational mapping of the DNA and RNA data, we expect to generate many large databases of information on expression of genes and antibodies and how they change in ageing. We will publish several papers on the three objectives of the project. We may identify new products for the clinic, or provide new knowledge on therapeutic combination of existing products. We may generate new intellectual property, which we will protect with patents. (We will also engage in active dialogue with the public to inform people about our research and its relevance to ageing and healthspan.)

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

The earliest beneficiaries will be researchers working in related aspects of lymphocyte development, who will benefit from our databases to inform their studies. They will benefit from new understanding of key cellular signalling pathways and gene regulation mechanisms that enable B cell growth and development, and how they are altered in ageing B cells.

Researchers working on ageing in other systems will benefit from new knowledge on common ageing pathways, and ageing heterogeneity (how individuals age differently)

Researchers working on growth, metabolism and gene regulation in other tissues will benefit from our findings, which will be relevant for many tissues.

In the longer term, clinicians and immunologists working with ageing patients will have greater understanding of the characteristics of the antibody repertoire that change with ageing, and may have new tools to assess immune system health.

Vaccinologists may have new insights into ways to boost vaccine efficacy, in the general population and in older people.

Clinicians treating B cell and other leukaemias will benefit from new understanding of how genes are organised on different chromosomes in the nucleus, and how this positioning may predispose to leukaemias.

Once intellectual property is protected, commercial interests may have new therapeutic products to develop.

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

We will maximise the utility of the large datasets we will generate, eg by sharing data early and seeking collaborations on important discoveries that we do not have the expertise or resources to follow up. We will publish our research as preprints on bioRxiv in advance of submission to journals. We will publicise any unsuccessful approaches to the scientific community to avoid others wasting resources on these (eg published mouse models in which we discover a fault). We will present our results at scientific conferences before publication. We will secure any potential intellectual property by patent to protect UK commercial interests. We will do this early in order to avoid delays in publication. We will actively engage with appropriate commercial and clinical interests to progress our results towards the clinic in a timely fashion.

Species and numbers of animals expected to be used

- Mice: 7160

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 will use mice because the mouse immune system, in particular lymphocyte development, is extraordinarily similar to the human system. Furthermore, gene regulation and cytoplasmic signalling, our main investigation areas, are also very similar between mouse and human. Additionally individual genes can be effectively deleted in mice in specific tissues without affecting the whole animal. We will use two types of mouse models. First, we will study mice in which specific genes that we have shown

are important in ageing, have been deleted only in B cells, ensuring the mice remain healthy overall. These studies will be in mice aged 3 months (equivalent to a human young adult). Second, we will compare 3 month-old wild-type mice with wild-type mice that we will age up to 24 months (equivalent to a human of over 70 years). This will enable us to study defects in B cells that occur as a consequence of ageing.

In addition to the fact that the ability to delete individual genes in mice enables highly specific and detailed studies that are not possible in humans, we are also particularly restricted to using mouse models specifically for these studies, because, unlike human blood samples, it is very difficult to obtain human bone marrow samples for research.

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

A small number of female mice (<100) will be injected with hormones to increase production of embryos (superovulation) and a similarly small number (<50) will receive embryos via surgical or non-surgical procedures to establish new mouse strains. A very small number of male mice (<10) will be vasectomised to help with this.

For the vast majority of our studies, including both gene-deleted and aged mice, we will remove bone marrow and spleen from mice post-mortem, and purify and/or grow B cells from these samples. We will occasionally re-inject some of these purified B cells back into mice to determine how efficient they are at restoring B cells in the mouse.

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

Superovulation, receipt of embryos and vasectomy described above are surgical procedures that are considered moderate, due to pain post-surgery. This is controlled by analgesia before and after surgery.

For most of the mice, we do not expect any adverse effects in our extremely high quality animal care facility. Some mice will be immunodeficient, but we know from experience that this has no adverse impact on health in our specific pathogen-free (SPF) facility. We also know that the vast majority of our aged mice remain healthy throughout the duration of our experiments. A small minority get tumours, but regular checking by our experienced animal technicians ensures these are detected early, and the mouse euthanised immediately.

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 expected severity for most of the mice will be mild. The expected severity for embryo recipients and vasectomy will be moderate. Together, the proportion of those in the moderate category will be 1% of the total mouse cohort.

What will happen to animals at the end of this project?

- Used in other projects
- Killed

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?

We need to use animals primarily because this is the only way that we can study the role of individual genes in detail, by investigating the effects of single gene deletion on all the stages of development they affect, in this case bone marrow B cell development. Furthermore, it is extremely difficult to study B cell development in the bone marrow in humans, because it is very difficult to obtain human bone marrow samples, in particular from healthy donors.

Nevertheless, as part of our current PPL and future plans, we have established a pilot human bone marrow culture system, which will replace some of our mouse studies and/or provide important cross species validation.

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

We have considered using mouse bone-marrow derived B cell lines.

Why were they not suitable?

These lines only represent one stage of B cell development at a time, and thus we can't study how all the stages of development affect each other. Also the culture conditions they grow in do not allow all of the possible different antibodies to be made, and some cell lines only allow one type of antibody to be made, so we could not investigate the role of individual genes in generating the millions of different antibodies that our millions of B cells make.

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 from our previous studies using aged mice, and mice in which we have altered a gene. This 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.

Data from previous experiments has enabled us to estimate the numbers of cells we will need for each experiment, and consequently the numbers of mice of each genotype.

Important considerations have included adjusting for the reduced numbers of B cells produced by aged mice compared with young mice.

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

We have consulted in depth with our Biostatistician, and have built on our previous experience of working with these and similar mice. In particular, we have learned that individual aged mice have much more variation in the genes they express compared with young mice. Accordingly we have had to increase the number of aged mice compared with young mice within some experiments, but this will reduce numbers in the future, since individual experiments will be statistically significant.

We have increased the focus of our experiments towards the B cell culture system that we have developed, instead of using ex vivo purified B cells. While culture systems have some caveats, and are not suitable for all experiments, they have the advantage that cell numbers can be expanded to provide many more cells per individual mouse, which also has the synergistic advantage that parallel experiments can be conducted in the same mouse rather than in separate mice, increasing statistical robustness. One mouse can provide the equivalent number of B cells expanded in culture to the number of ex vivo purified B cells from 20 mice. We have used the NC3R's Experimental Design Assistant to ensure we are considering all relevant aspects of design.

We will avail of ongoing improvements in next generation sequencing methods that have reduced cell numbers required. For example our capture HiC method previously required 10 million cells, but improved methods now only require half a million cells.

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

Our mouse facility has an extremely efficient pipeline of 10 day 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 the desired type of cells. We will do pilot studies with cre recombinase expressing mouse lines crossed to mouse lines expressing conditional floxed genes of interest to ensure, first that the gene is efficiently deleted in the cells in which we want it to be deleted, and second that the approach does not delete the gene in other cells. For the latter we will make extensive use of genotyping for deleted genes, which has previously alerted us to off-target gene deletion events which necessitated re-design of our experiments. Pilot studies ensure minimum use of mice before decision points are reached in experimental design.

For our aged mouse studies, we have an established and highly successful programme of sharing of tissues with other investigators, which we will continue. This often includes up to 4 investigators using

tissues from the same mouse.

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.

We will use wild-type, transgenic, gene-targeted young mice up to 3 months, and aged mice up to 26 months.

Transgenic and gene-targeted mice usually only have a single gene, or at most, three genes, altered. Either by natural expression patterns, or by targeted deletion this only affects a small number of tissues. In this project these are primarily bone marrow and spleen B cells. Any potential pain or suffering would arise from infection, since these mice will have defective B cells and thus be more susceptible to infection. All mice are housed in very clean conditions, which prevent infections, and thus prevents any such pain or suffering.

Most experiments only take tissues post-mortem. A small number of experiments will involve injecting bone marrow cells into a recipient mouse to reconstitute the immune system. These experiments provide detailed in vivo knowledge on the ability of haematopoietic cells from a gene targeted mouse to contribute to B cell development, in ways that studying cells ex vivo can not do. Furthermore, relatively few cells from a single gene-targeted mouse can reconstitute several recipient mice, contributing to greater statistical power. In some cases these experiments will restore the immune system of immunodeficient mice, and will have no negative impact. In a small number of cases, wild-type mice will be irradiated to remove endogenous lymphocytes, to ensure donor bone marrow is providing reconstitution. This can occasionally lead to ill health, but these mice will be carefully monitored and any animal exhibiting pain or distress will be culled immediately.

Thus in most cases, there is no pain, suffering, distress, or lasting harm expected with the models or planned experiments. The exception to this is that a small number of animals that will be embryo recipients, or will be vasectomized, will experience transient pain due to surgery. This will be ameliorated by analgesia.

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

We can't use animals that are less sentient, because they do not have an adaptive immune system that is comparable to the human system. This system first appears in evolution in the lamprey and zebrafish, but in a very rudimentary form, that is not suitable for in-depth studies. Species including C elegans and Drosophila melongaster, have rudimentary innate immune systems, but do not produce lymphocytes.

We also cannot use early stage mouse embryos because the adaptive immune system does not develop until late stages of embryogenesis.

Although some species including C elegans are established models of ageing, their lack of an adaptive immune system makes them unsuitable for our studies.

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

We are mindful of increased possibility of welfare costs as mice age, and we have implemented a programme of increased frequency of observation of these mice. We have developed a detailed checklist of possible age related changes, and procedures for monitoring and treatment, which informs our decisions. We will gather data from these studies to further refine best practice.

We will implement any refinements developed by our animal house staff, who have a long history of innovative practice, including environmental enrichment.

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

We will follow published best practice guidance from NC3Rs, LASA and the Home Office.

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

We will stay informed about advances in the 3Rs during this project via the monthly NC3Rs e-newsletter, NC3R workshops and webinars, and our annual Institute 3Rs seminar. We will avail of Home office advice made available to us through our dedicated Home Office Liaison.

To implement advances effectively, we will follow advice from our local AWERB. Our animal facility also has a dedicated Strategy Committee and a User group, on which we are represented. Both groups discuss and make collective decisions about advances in the 3Rs and advise on how they can be implemented, both across the organisation, and by individual groups.