Home Office

NON-TECHNICAL SUMMARY

IN VIVO STUDIES OF PATHWAYS AND CELLS INVOLVED IN DETECTING INFECTION, DAMAGE AND CANCER

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

Dendritic cells, Immunoregulation, Immunotherapy, Autoimmunity

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The fundamental objective is to understand how the immune system "decides" how to react to antigen challenge.
The detailed objectives can be summarised as:
1) Which signals and pathways activate dendritic cells (DC) and how are they integrated?
2) Do all signals and pathways lead to the generation of "effector" DC with similar properties?
3) How do different DC subsets develop, what are their properties and do they respond to distinct activation signals?
4) What are the consequences of differential DC activation and DC heterogeneity for adaptive immunity and tolerance?
5) How can DC activation be manipulated to control the adaptive immune system?

A retrospective assessment of these aims will be due by 05 August 2024

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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.

What are the potential benefits that will derive from this project?

Cell-mediated immunity holds promise in diseases such as cancer, HIV infection and malaria where antibody-based immunotherapies have failed to deliver clinical benefit. Cell-mediated immunity to cancer is, effectively, a T cell response. The success of cancer immunotherapy, therefore, depends on our ability to prime T cells specific for tumour antigens and to steer their differentiation into effector cells capable of tumour destruction. Priming and directing T cell responses is the principal function of dendritic cells (DC), the major class of antigen-presenting cells (APC) in the body. Despite appearing as a basic research programme, this work has the potential to lead to design of better vaccines and immunotherapies for both infectious disease and cancer and to the development of immune deactivation strategies for autoimmune disease.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Over the course of a five-year study we anticipate that we will require up to 75,000 mice and 50 rats to undertake a project of this scope.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The vast majority of animals bred and used under this Licence are expected to have a lifetime experience equivalent to that of their wild-type background strains. Genetically altered mice are also expected to develop the same range and incidence of known strain-specific health conditions as wild-type individuals. For example, wild-type C57BL/6 · mice have reported incidences of ophthalmic abnormalities such as microphthalmia of between 4.4%-10% and are prone to hydrocephalus and dermatitis. We expect most of the genetically altered strains used in this study to exhibit similar pre-weaning losses and display rates of adult mortality similar those of equivalent wild- type mice. We will monitor continuously for any significant increases in these rates.
However, approximately 25% of mice used under this Licence will be those that may present phenotypes with the potential exceed the mild severity classification. These will include such genetically altered mice as those with immunodeficiencies, a predisposition for autoimmunity or for tumour development, and those wild-type mice where such conditions are induced experimentally. Throughout this Project Licence the FELASA and NCRI guidelines, will be used to define severity categories objectively.
Any individual mouse will typically undergo only a very limited number of the optional steps available and it is not anticipated that cumulative adverse effects will result from any combination of such steps. However, as it is not possible to fully predict the nature or severity of all potential adverse reactions for all types of mice undergoing novel combinations of procedures there will be careful monitoring for possible side effects. For animals exhibiting any unexpected clinical signs, such as piloerection and an intermittent hunched posture for 24hrs the humane endpoint will be deemed to have been reached and the animal will be culled, otherwise at the end of any protocol all animals will be humanely killed.

A retrospective assessment of these predicted harms will be due by 05 August 2024

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Research into the cellular and molecular interactions that determine the outcome of antigen challenge requires an intact immune system. Therefore, by definition, such research cannot be carried out in vitro. The development and function of the immune system involves many different cell types interacting in a dynamic environment. For example. the progression of an infection within a whole organism involves changes of antigen expression and presentation that evolve with both time and spacial distribution. Similarly, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding cells, governed by multiple signals
originating from both their immediate neighbours and from distant tissues. These factors combined with the involvement of multiple host cell-types and the clonal expansion and migration of effector cells mean such research cannot be carried out in tissue culture alone and can only be addressed by the use of animals. The mouse is one of the model organisms that most closely resemble humans. The human and mouse genomes are approximately the same size, and display an identical number of genes, which are functionally conserved. Further, mice have genes not represented in other animal model organisms (e.g. nematode worms and fruit flies) such as those involved in the adaptive immunity. Mice can be genetically altered, there is extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field

A retrospective assessment of replacement will be due by 05 August 2024

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

We will collect as much evidence as possible from current literature, and through the analysis of available data. We will also perform studies in vitro using established cancer cell lines and mouse primary .non-transformed cells. These studies will precede and guide the generation of relevant transgenic mouse models. We will minimise the number of animals by mostly using inbred mouse strains, and by housing them under identical conditions to limit variability. We will avoid overbreeding, and lines under sporadic use will be maintained at low levels, and frozen whenever practicable, and/or maintained in collaboration with other licences to minimise redundant breeding. The proposed experimental designs and methods of analysis will be discussed with members of the laboratory, and those of our collaborators, and we will seek additional advice from the statisticians employed by our Institute. We will perform pilot experiments for comparing genotypes using small numbers of animals per group. If some effects are worth investigating further we may perform larger cohort studies to determine if the observed difference is statistically significant. The size of the cohort will depend on the observations made from the pilot studies, and will be determined using power calculations. We aim to use the minimum number of mice per group that will be informative.

A retrospective assessment of reduction will be due by by 05 August 2024

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The use of genetically identical mice in the field of immunology not only reduces experimental variability but also eliminates immunological incompatibility when cell transfers are carried out between various knockout, transgenic and wild-type strains of mice. Without such a defined genetic background nearly all of these experiments would be impossible.
In addition, we use specific genetically-modified animals to understand the molecular events and steps involved in the immune activation process or as a way to direct immune responses against defined model antigens, thereby making analysis and quantitation of immunological effects easier.

The categories of genetically modified mice necessary for achieving the objectives of the project include;
i) Strains expressing transgenes which play a role in, or with expression targeted to cells involved in, immune function and regulation.
ii) Strains with the absence of, or modifications to, genes involved in both the innate and adaptive immune system, examples include pattern recognition receptors, components of signalling pathways, lymphocyte surface markers and receptors.
iii) Strains expressing DNA recombinases and/or reporter genes of such recombination.
iv) Strains expressing oncogenic transgenes that increase the incidence of spontaneous tumours.
v) Strains developing (or with an increased tendency to develop) spontaneous autoimmune or inflammatory conditions due *to* transgenic modification or mutation.
vi) Crosses of such strains.
Whenever possible we will generate transgenic mice in which mutations are induced specifically at certain times or places where mice should not display a phenotype until the mutation in the candidate gene is induced. Where the immune status of the animals might compromise health, they will be maintained in isolators or IVCs (individually ventilated cages) under barrier environment, to avoid infections. In our experiments we will set clear humane endpoints and will for each and every experiment, as part of good laboratory practice, write an experimental protocol, which will include details of possible adverse effects. These experimental protocols will be provided to all the staff involved in the experiment.
In addition, when considering which route of administration of substances to employ, we will strive to use the least invasive route whilst maintaining direct control of dose. The choice of route to administer a substance or cells will be such as to achieve "best practice", i.e. to minimize or avoid adverse effects, reduce the number of animals used, and maximize the quality and applicability of results. For that reason we propose in this project licence a variety of routes of administration of substances and cells to achieve the scientific objectives. Although in the majority of cases we will primarily use standard routes of administration such as intravenous or intraperitoneal injections, the active concentration, volume,

stability, and toxicity of a particular substance or cells may require administration through a non-standard route such as injection adjacent to or directly into a tumour. For all procedures coded (AB) or (AC), general or local anaesthesia as appropriate will be induced and maintained using agents and routes of administration suitable for the species and the nature and duration of the procedure.

A retrospective assessment of refinement will be due by by 05 August 2024

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?