Home Office

NON-TECHNICAL SUMMARY

Regulation of immune response during infection and inflammation in rodents

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.

Key words

Immuneregulation; immunepathology; infection; autoimmunity; inflammation.

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
- Required at inspector’s discretion

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 immune system has developed to be highly specialized and effective in eradicating a wide variety of pathogens. The adaptive arm of the immune response, consisting of antigen-specific T and B cells interacts with innate immune cells to mediate an effective response to infectious pathogens. This interaction is tightly controlled by many mediators to achieve an appropriate response with minimum immunopathology. The adaptive immune response is enhanced upon secondary exposure (memory) as in vaccination, but in tuberculosis (TB) this is ineffective. Common to infections such as TB a chronic, persistent infection follows, and their control is mediated primarily by T lymphocytes and innate cells that activate T cells, which can be suppressed by the pathogen. In order to protect or cure individuals against infectious agents causing diseases such as tuberculosis (TB), a disease of major morbidity and mortality in man, particularly when antibiotics are ineffective, an understanding of the immune response is badly needed to intervene and induce immune enhancers and/or immune modulators, to achieve maximum protection with minimum pathologies. Moreover, the same molecules/immune modulators and/or enhancers that bring about protective immune responses against a pathogen, may also result in immune and autoimmune pathologies, such as rheumatoid arthritis (RA) or multiple sclerosis (MS), or inflammatory pathologies such as asthma. This, and the multigenetic complexity of inflammatory and autoimmune disorders has made therapeutic intervention in these diseases also very difficult, and many of the current drugs have multiple side effects. Likewise, immune responses required for the clearance of pathogenic micro-organims can sometimes result in immune damage to the individual. Molecules that protect against immune damage, like IL-10, on the other hand, can contribute to chronic infection. Likewise, certain genetic deletions, including that of Il10 or mutations in genes which regulate IL-10 may affect the control of the immune response to infection or to intestinal microbiota or pathobionts (microbes that cause gut inflammation) and lead to disease.

We aim to understand the immune molecules that lead to a balanced immune response so as to test novel strategies of preventive and therapeutic immune intervention. First,we will identify molecules/pathways leading to over-exhuberant responses, which may result in disease during infection, inflammatory or autoimmune diseases, or cancer, and identify molecules that regulate them to prevent immune pathologies. Second,we will identify immune molecules resulting in protection or chronicity during infectious diseases in mouse models refining them to more accurately reflect the human counterparts. We will identify mechanisms by which pathogens such as *Mycobacterium tuberculosis* and other bacteria, viruses or parasites, act to subvert these responses, and how certain infections lead to over-exuberant responses and host damage. Using this knowledge we aim to identify therapeutics to control infections.

A retrospective assessment of these aims will be due by 29 October 2023

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?

An understanding of the molecules enhancing and regulating the immune response, may help to design therapeutics in order to manipulate inflammatory and autoimmune disorders specifically, effectively and with minimum side-effects such as the development of chronic infectious diseases. In addition, this could lead to discovery of immune modulators to protect against chronic infections. To achieve both these goals we will require a very thorough understanding of the molecular basis for the regulation of the immune response. Hence, this research area is still highly active internationally and our laboratory is actively pursuing this line of research to understand the immune response to identify novel molecular and cellular events and novel or immune modulators, to protect against inflammatory, autoimmune and infectious diseases.

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?

We plan to use wild type and genetically modified mice for in vitro and ex-vivo experiments; and for in vivo experiments in mouse models of immunomodulation, infection; inflammation; allergy; autoimmunity; and cancer – the main emphasis will be on infectious disease models but for understanding mechanisms of immune regulation the other models are employed, albeit to a lesser extent. Experiments will range from 1 – 3 days for certain infections; to a maximum of 180 days for others (mainly TB models). Over the five years we anticipate breeding and maintaining 30,000 genetically altered animals (with or without associated wild types); 1000 for phenotyping, tissue provision and long-term monitoring; 5000 for experiments in vivo for defining mechanisms of immune modulation in innate and adaptive immunity and in allergic,
inflammatory and cancer mouse models of disease; 10,000 in models of infectious diseases to determine protective yet regulated pathways; 500 in autoimmune disease to define immunomodulators.

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?

Genetically modified animals with mutations in molecules potentially involved in the immune response will be used throughout the course of the project license. Many of them may be immunocompromised, and hence will be kept under specific pathogen free conditions. Furthermore, they will be monitored

from birth so that any defects leading to mouse discomfort may be stopped by killing the animal. In some cases where therapeutic molecules are required to be tested these mice may be maintained alive for the minimum duration required for such intervention and/or analysis.
We anticipate that breeding and maintaining 30,000 genetically altered animals (with or without associated wild types) will be of mild severity; 2500 mice are approximated to reach severe signs (2000 through infection; 500 through autoimmunity); 6000 to reach moderate severity (through infection) and 5000 to reach moderate severity (through immune modulation); 2000 to reach mild severity (infection). All mice will be strictly monitored within each protocol to ensure that the defined severity is adhered to.

A retrospective assessment of these predicted harms will be due by 29 October 2023

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.

Firstly, this work will include *ex vivo* studies using immune cells obtained directly from either normal or genetically manipulated mice, to dissect the mechanisms underlying the activation and effector function of immune cells *in vitro*. This approach cannot be replaced by cell lines that have been maintained in long term culture, since they invariably do not maintain their true fidelity. Although this may result in the use of large numbers of mice, this first step together with findings we are making in clinical studies will **replace** the initial need for *in vivo* manipulation of mice. Such *ex vivo* studies may reveal the molecular basis for enhancement or suppression of immune responses. However, these findings will still need to be verified *in vivo*, in whole organisms where multiple complex interactions take place resulting in the overall response.

A retrospective assessment of replacement will be due by 29 October 2023

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.

For most of the experiments quantitation is required and 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). Pilot experiments will use between 5-8 mice per group, which should be sufficient if a significant result is obtained and experiments will be designed to use the minimum number of mice that

will provide statistically reproducible results which are set using power analysis, generally using a significance level of 5%, a power of 80% and at least practicable difference between groups of 20%. Once a desired effect has been obtained it may be necessary to use a greater number of mice per group in order to facilitate obtaining rare immune cells involved in the response for function analysis.

A retrospective assessment of reduction will be due by by 29 October 2023

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.

We will use the laboratory mouse as the model organism. The mouse is the best characterised model for these studies, with many features applicable to human infection. Their immune responses are well defined and the technology enabling sophisticated manipulations of the haematopoietic and immune system is highly developed. Mouse transgenic and knockout techniques are well established; mice have a relatively short generation time; the haematopoietic system of the mouse has been extensively studied and, in addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this project to the same extent as the mouse. All mouse models used will be assessed such that the severity will be reduced to the minimum in terms of infection or inflammation burden required to show effects and obtain meaningful results. Mice will be monitored closely to ensure that the numbers are maintained at the minimum severity possible to obtain meaningful results that may inform our knowledge to advance therapeutics.

A retrospective assessment of refinement will be due by by 29 October 2023

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?