NON-TECHNICAL SUMMARY

Molecular mechanisms of tolerance and immunosuppression

Project duration

5 years 0 months

Project purpose

- (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

Immunity, Inflammation, Infection, Cancer

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

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

What is the aim of this project?
The normal function of the immune system is required to fight infections by bacteria and viruses while its disordered function contributes to numerous disease processes including autoimmunity, allergy, chronic infection and cancer. The immune system is composed of diverse cell types that can either promote or inhibit immune activation. While inhibitory components of the immune system are required to suppress autoimmune and allergic inflammation in a process referred to as immunological tolerance, they can also suppress potentially beneficial responses against infections and cancer, in a process known as immunosuppression. Importantly, newly developed therapies targeting mechanisms of immunosuppression have shown promise in activating immune function in patients with cancer, but since the mechanisms of immunosuppression being targeted also contribute to immunological tolerance, a proportion of individuals treated develop inflammation which causes side effects and limits therapy. Our research aims to discover new mechanisms underlying immunological tolerance and immunosuppression, and define those mechanisms that have distinct rather than shared roles in these two processes. Discovery of such distinct mechanisms may enable more specific therapies to be developed, allowing, for example, disruption of immunosuppression without induction of inflammatory disease in patients.

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?

Therapies that work using the immune system have brought about significant health benefits on a global scale. For example, therapies targeting immunosuppressive mechanisms within the tumour are presently revolutionising the treatment of certain chronic diseases such as cancer. Our research will extend our fundamental knowledge of how the function of the immune system is controlled not only to prevent otherwise deleterious autoimmune and allergic inflammation but also to limit effective immunity against chronic infections and cancer. The research will also provide a basis for development of new therapies aimed at controlling immune function in patients with a variety of disorders in which the immune system plays a critical role, including cancer, inflammatory diseases and infections.

Our research will also be of benefit to researchers in related academic fields studying inflammatory diseases, infectious diseases, ways in which cells control their gene expression and tumour biology. Aside from its relevance to academic researchers, the work is relevant to researchers aiming to make new therapies for individuals with immune-mediated disorders, cancer and chronic infection, including pre-clinical researchers and the pharmaceutical industry, with whom we have established collaborations.

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?

About 50680 mice are expected to be used over a five year period. The immune system in mice is similar enough to the immune system in humans that valuable parallels can be drawn. The availability of different genetically modified mice and reagents that recognise mouse cells means that this species can be used more efficiently than any other species to ask questions about the role of particular genes in the immune system. The breeding of the mice will be planned and monitored carefully to ensure that
we only produce the mice needed for experiments. The majority of the mice will be used to provide immune organs harvested for lab-based assays. Other mice will be immunised, infected or will be challenged with tumour cells, and the results immune response monitored. In each case, the lowest number of mice that produce robust reproducible (statistically significant) results will be used.

**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 majority of mice bred under this license will have genetic alterations that allow the function of molecules within the immune system to be tested. Animals are housed under tightly controlled conditions where their exposure to infections is limited. In many cases, animals with genetic alterations will not have any abnormalities and will be killed humanely and their tissues used for experiments in the laboratory. Where it is necessary to gain new knowledge about how the immune system works within living organisms, some mice will be subjected to immunological stimuli. For example, we will test the immune response of animals to tumours by implanting small numbers of tumour cells under the skin of animals and letting them grow. Tumour growth will be monitored regularly by trained staff and animals will not be allowed to suffer excessively with euthanasia of animals triggered by well-defined criteria. Euthanasia is often carried out by administering carbon dioxide gas to animals which causes them to become unconscious and die after five minutes of exposure. After this, a secondary method of killing, such as dislocation of the neck is used to ensure that animals are dead. After euthanasia, tumours will be analysed in the laboratory to gain insights into how tumour immune responses are suppressed. In general, these experiments take around three weeks from tumour injection to euthanasia. Because some of the ways in which immunity to tumours is suppressed are similar to the ways by which the body prevents inflammation, we also need to use models of autoimmune and allergic inflammation and also to test immune function under normal conditions and in animals reconstituted with immune cells. For example, animals will be administered a substance that causes gut inflammation in the drinking water. Animals will be routinely assessed for signs of illness and weight loss and animals likely to experience excessive suffering or weight loss will be euthanised prior to onset of such disease. Such experiments usually take two weeks from the administration of the colitis-causing substance to euthanasia. In each case, the exposures given to animals will be adjusted to elicit measurable and informative responses but to minimise pain, suffering, distress or lasting harm. Humane endpoints have been carefully considered to prevent excessive suffering, are clearly documented and available to trained staff who regularly monitor experimental animals. As a result, no animals are expected to die as a result of experimental procedures. Experimental protocols are continually refined to incorporate new knowledge and technologies that reduce harm.

**Replacement**

**State why you need to use animals and why you cannot use non-animal alternatives.**
Animals are necessary to understanding how the immune system works because the various interactions of immune cells with other cells and substances in the living animals cannot yet be generated in test tubes. Features such as the distribution of immune organs throughout the body and the ability of immune cells to migrate into almost all tissues of the body make investigations of the immune system in the whole animal context essential. Adaptive immunity (the type of immunity which remembers previous exposures), which is the subject of this research, evolved in vertebrate animals and is not present in less sentient organisms. Among vertebrates, many cellular and molecular features are highly conserved between mouse and man. Many useful tools and well-established experimental models for experiments in mice already exist, enabling us to perform research using mice in an efficient manner that minimises the number of animals we need to use. Therefore use of mice in this research is necessary.

However, where possible, before using animals in experiments, we use cell culture experiments using immune cells in petri dishes, to determine whether certain molecular pathways are likely to have an important role in controlling the immune system. These experiments replace the need to use animals at the early stage of discovery. However, it is often necessary to validate findings made in the petri dish using experiments in mice.

Reduction

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

We have implemented changes to reduce the number of animals we need to do this research. This includes improvements to the efficiency of generation of genetically modified mice as well as breeding and mouse colony management. We carefully calculate the size of the groups of mice used in experiments so that enough numbers are used, but not too many, to make statistically sound conclusions from our experiments. Finally, by generating and publishing so-called ‘high-content’ data, or data that contains a lot of information from single samples, we reduce the need to do repeated experiments to make single different measurements frequently.

The use of longitudinal non-invasive in vivo imaging as a means as a surrogate marker of the migration and frequency of cells will enable multiple time course measurements to be obtained over time from the same animals, enabling us to gain more information from the same number of animals.

We will seek to further improve experimental design with careful consideration applied to make sure we use the least number of animals required to test scientific questions.

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.
All experiments we will do are classified as causing only either mild or moderate suffering to animals. We have defined clear clinical endpoints that will trigger the end of an experiment for a particular mouse or a cohort and as a result, death of animals is not an endpoint in any experimental system used. However, careful monitoring of experimental animals by trained staff anticipates deterioration in health and animals such that experiments are ended before animals are subjected to suffering in excess of that defined by either mild or moderate severity limits.