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NON-TECHNICAL SUMMARY

Immunology and immunopathology of malaria

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

malaria, Plasmodium, immunity, immunopathology, virulence

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?

Malaria is a disease that kills approximately 400,000 children a year in sub-Saharan Africa alone. We still do not know how the immune response is regulated in a malaria infection, nor the key components involved in protective immunity, immunological memory and immunopathology.

Rodent malaria parasites in laboratory mice are very good models to dissect these mechanisms, as they have many of the features of human malaria. The severity of malaria is determined by interactions between molecules of the malaria parasite with the host. Therefore, it is important to identify the parasite molecules causing virulence and the nature of impact they have on the host.

P. falciparum malaria in children is often present as a co-infection with other pathogens, and also can be associated with a B-cell cancer, Burkitts lymphoma. The exact mechanisms of the interaction of the malaria parasite and co-infecting viruses with B-cells to influence development of lymphoma are not known. We will use the knowledge of the B-cell response to investigate how malaria interacts to induce lymphoma development.

The *Plasmodium* components responsible for stimulating host responses are not fully understood. It is thought the large multigene families of the parasite are involved in these processes. We aim to elucidate the role of a large multigene family called pir in our mouse model. This gene family is shared by all species of *Plasmodium*, and thus our rodent model will provide useful information on their role in human malaria.

A retrospective assessment of these aims will be due by 25 April 2025

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?

The potential benefits that will derive from this project include a detailed knowledge of the protective and pathological immune responses induced by infection with the malaria parasite, and the conditions necessary to induce long-lasting immunity. Furthermore, we will generate important information on how malaria impacts on the development of a lymphoma, as a model for understanding the relationship between Burkitt's Lymphoma and malaria in children. This knowledge can be harnessed to develop effective vaccines or interventions. Understanding which parasite components are responsible for a virulent infection and how they act in the host gives us information that is transferable to humans, and will allow us to design effective interventions to reduce mortality and severity of malaria in humans.

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?

Mouse.

For the research projects of 12-15 researchers approximately 17,650 mice will be used over 5 years. Approximately 30% of these are in the breeding programme and will not be subject to any procedures.

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 (60-70%) infected with *Plasmodium chabaudi* will not exceed the moderate limit. Most mice recover from moderate symptoms within 48hrs; however, if mice are still displaying a clinical score of 3 or more for 48 hours they will be culled by a Schedule 1 method or exsanguinated under terminal anaesthesia immediately. Clinical scores are determined as follows: (1 point for each of the following signs displayed by a mouse): Marked staring coat, anaemia, hypothermia, hunched posture where the animal adopts normal posture when provoked, subdued behaviour even when provoked, reduced peer interaction, shivering, moderate respiratory signs (altered or noisy respiration, increased respiratory rate at rest).

In the minority of experiments where we need to establish the link between morbidity and mortality in order to gain better end point and prognostic criteria, and to uncover the causes of pathology or the mechanism(s) of immunopathology, symptoms induced by the infection may reach severe (and the mice could potentially die). In these cases if mice display a clinical score of 4 or higher for 48 hours they will be culled by a Schedule 1 method or exsanguinated under terminal anaesthesia immediately. Clinical scores are determined as follows: (1 point for each of the following signs displayed by a mouse): marked staring coat, anaemia, hypothermia, persistently hunched posture, lack of appetite, inactivity, unresponsive behaviour to extraneous activity or provocation, clinical signs of suffering like persistently laboured respiration (dyspnoea), or persistent diarrhoea.
If a single sign is shown animals will be monitored more closely. Monitoring during this time will be done in conjunction with animal care staff to ensure that the most appropriate action will be taken.

At the end of an experiment mice will be killed by a Schedule 1 method or exsanguinated under terminal anaesthesia.

A retrospective assessment of these predicted harms will be due by 25 April 2025

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.

For breeding and maintenance of the vector mosquitoes, females require blood meals for egg production. We have replaced mice as the source of blood with commercially sourced horse blood.

It is not possible to culture any stage of rodent parasites *in vitro*. The complex immunological interaction between the malaria parasite and its host cannot be reproduced in cultures or in animal of lower sentience.

A retrospective assessment of replacement will be due by 25 April 2025

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 human malaria data. This will precede and guide the generation of relevant transgenic mouse models.

The breeding of transgenic animals will be reduced through collaborative access to strains. 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.

For each experiment, we will seek advice from the statisticians employed in my laboratory and/or in the Institute. We will design experiments using agreed guidelines (PREPARE) to obtain significant findings with the minimum number of animals.

We will perform pilot experiments in which a small number of animals per group are used for comparisons. Depending on the results obtained from pilot studies we will then proceed to perform larger cohort studies to determine if the observed difference is statistically significant.

Furthermore, we will use, whenever possible, modified bone marrow cells for the reconstitution of the immune system in host animals, which permits the increase of sample measurements together with the

reduction of the breeding of transgenic animals. This approach also allows bypassing complex genetic crosses aiming to identify intrinsic versus extrinsic phenotypes.

A retrospective assessment of reduction will be due by by 25 April 2025

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 mouse is one of the model organisms that most closely resembles humans, and many of its genes are functionally conserved. We have extensive knowledge of the mouse immune system and have reagents to define immune responses. In addition, there are many genetically altered mice available that allow us to ask whether defined components of the immune response are necessary for control of the parasite or for inducing pathology.

Knowledge of the mechanisms leading to malaria disease and immunity can only be dissected in animal models. We have refined our model to use mosquito transmission as far as is possible, which most closely resembles the human infection. This has substantially reduced the severity of the blood-stage infection, and results in lower parasitaemias, less anaemia, smaller drops in temperature and little to no loss in body weight in most inbred strains of mice. These all fall within the “moderate” range. Therefore, most procedures in this protocol are now designated “moderate”. However, in immunodeficient, genetically altered or mutant mice, and particularly in splenectomised mice, infections are likely to be more virulent. We will monitor as follows to minimise suffering: If animals display a marked staring coat and anaemia plus additional signs of persistently hunched posture, anaemia, shivering, lack of appetite, inactivity, unresponsive behaviour to the environment, or provocation, and/or clinical signs of suffering like persistently laboured respiration, or persistent diarrhoea for a period of 48 hours they will be culled using a schedule 1 procedure. If a single sign is shown animals will be monitored more closely. If 4 or more signs are present for 48 hours, animals will be culled by a Schedule 1 method or exsanguinated under terminal anaesthesia immediately. Monitoring during this time will be done in conjunction with animal care staff to ensure that the most appropriate action will be taken.

We expect that from this proposed study we will define better predictive markers of malarial disease and virulence, which will be immediately incorporated into our experimental work.

A retrospective assessment of refinement will be due by by 25 April 2025

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?