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G: NON-TECHNICAL SUMMARY (NTS)

Please attach the Non-technical Summary as generated by your application in ASPeL.

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals).

Word limit; 1000 words

Project Title	Determining important regulatory pathways that control immune responses to infection
Key Words	immune system, infection, parasite, bacteria, virus
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes (a) basic research;

(b) translational or applied research with one of the following aims:

Yes (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Yes (ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

No (iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

No (c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

No (d) protection of the natural environment in the interests of the health or welfare of man or animals;

No (e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;

No (f) higher education or training for the acquisition, maintenance or improvement of vocational skills;

No (g) forensic inquiries.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Our immune system must be activated to deal with dangerous pathogens that enter the body, but at the same time be regulated so it doesn't attack our own tissues or harmless substances. Failure of this regulation can result in devastating immune-mediated disease. Therefore, understanding the cells and molecules that regulate the immune system in health and infection is crucial in identifying potential targets for therapy in immunological disorders.

Our project will focus on the cells/molecules that regulate immune responses to infection. Specifically, we use knockout and transgenic mice to identify how particular cells/genes in regulating the immune system during infection with viruses, bacteria and parasites. Our project aims to identify cellular and molecular pathways that prevent appropriate immune responses that can be targeted to promote beneficial responses during infection.

Additionally, we aim to discover pathways that promote so-called 'immunological memory', the process by which our immune system remembers a previous infection and responds more efficiently if we are infected with the same pathogen again.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Our work aims to identify pathways that boost protective immune responses when we are infected, and prevent unwanted self-harmful immune responses. Such pathways may be targets for therapy in the future, to enhance pathogen clearance and reduce associated tissue damage. Our work will also identify pathways that are beneficial in promoting immunological memory, thus providing targets to promote efficiency and function of vaccines.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use mice, and estimate that approximately 12,500 mice will be bred during the 5 year project, with 10,000 mice used in procedures.

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

Our work will involve using models of parasite, bacterial and viral infection.

Parasite models: Infection is generally without symptoms or with only transient moderate suffering to the mouse. Parasites are either expelled, or develop into a chronic, symptomless infection.

Bacterial models: Mice will develop acute infection and some illness (e.g. weight loss, lethargy). For some pathogens used, this is transient and illness will fully resolve (moderate severity). Some pathogens will cause severe infection after ~1 week, and mice will be monitored closely and culled immediately if symptoms reach a pre-determined threshold. This severe procedure is wholly necessary for our project, as it will allow us to determine important interventions that can alleviate symptoms of infection, thus identifying potential therapeutic targets for treatments of severe bacterial infection.

Virus models: In some viral models, mice will develop acute illness peaking ~1 week after infection. However, we will only use doses that mice are known to fully recover from, with return to health ~2 weeks post-infection.

In some experiments, after animals have fully recovered from initial infection, they will be re-infected to identify cells and molecules that regulate 'immunological memory'- the process by which we respond better and faster to infection the second time round.

At the end of procedures, all animals will be humanely culled.

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Application of the 3Rs

Replacement

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

Replacement

The use of animals is imperative to the success of the project. The mammalian immune system is complex, with many different cells and molecules working in combination to produce a co-ordinated response. Thus, using lower organisms such as *Drosophila* or zebrafish is not feasible, as they do not possess a complex immune system seen in mammals. Similarly, *in vitro* models cannot give an accurate reflection of the cellular and molecular complexity of the mammalian immune system. Thus, use of mammals is essential, with mice proving an invaluable tool in studying immunity for the past 40 years.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

From the outset of our project, we will consult with specialist statistical consultants contracted by the University to provide advice on designing experiments and statistics. Such advice will allow us to use the minimal possible mice to achieve statistically significant results.

All data analysis will be conducted according to a pre-specified plan drawn up with the statistical consultants, with statistical tests performed with their input.

Refinement

Explain the choice of animals 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.

Refinement

Choice of species, models and methods.

As stated above, given the complex organisation of the mammalian immune system, it is unfortunately not possible to recreate these conditions using lower organism models (which do not contain a complex immune system) or *in vitro* cell models. Thus, the use of mice is crucial in the study of mammalian immunity. There are numerous examples of discoveries made in mice that have led to the direct identification of similar systems in the human immune system, with such cells and molecules now being clinically targeted in disease.

There may be opportunities to perform more focussed *in vitro* experiments if, during the course of mouse experiments, we identify cell types that are directly affected during infection. We would then isolate these cells and determine their responses to different parasite/bacterial/viral products, and how different molecules/pathways affect their responses to pathogens.

Minimisation of animal suffering

Intestinal parasite infection models

All intestinal parasite infections are well established models of infection used by researchers over many years, with the majority causing no detectable suffering or distress to the animal. In a minority of cases (e.g. infection of mice with *Toxoplasma gondii*) mice will develop acute inflammation during infection. Here, animals will be closely monitored (with frequency increased leading up to time points

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where inflammation has been previously shown to occur) and should any unreasonable loss in condition be observed, the animals humanely killed.

Viral infection models

The viral models used will result in short-lived infection with moderate weight loss and illness, but mice expel the infection and fully recover from symptoms. However, all mice will be monitored closely in peak times of infection (3-7 days post-infection), and if any unreasonable loss of condition is observed the animals humanely killed.

Bacterial infection models

Some bacterial infection models (e.g. *Francisella tularensis* LVS) cause a severe acute infection. It is important for this level of infection to be reached, to determine cells/molecules and interventions that reduce the symptoms of infection. Thus, we need to reach a point in control animals where infection is established to determine whether any benefit has been achieved from gene/cell knockout. Other models (e.g. *Citrobacter rodentium*) cause modest weight loss and diarrhoea but resolve within 3-4 weeks with mice fully recovering.

In all experiments, we will closely monitor mice (with increased frequency of monitoring at time points close to when acute infection is expected to occur), and have a detailed scoring system in which to assess the health of the mice during severe acute infection. If an agreed level of discomfort is reached (based on a robust clinical scoring system), the mouse will be immediately humanely killed.