Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 7

Projects with a primary purpose of: Basic Research – Immune System

Project Titles and keywords

1. **Immunity against bacterial and paediatric diseases**
    - Vaccines, infectious diseases, paediatrics, bacteria

2. **Poxvirus virulence and immunogenicity**
    - Immunisation, vaccines, immunology, virus virulence, protein function

3. **Development and function of the immune system**
    - Lymphocyte, Infection, Autoimmunity, Signalling

4. **Genetic and physical control of B cell activation**
    - Antibody responses, activation of immune cells, signalling, endocytosis

5. **Immunity to ruminant endo- and ecto-parasites**
    - Vaccine Parasite Sheep Cattle

6. **Salmonella pathogenesis during asymptomatic Malaria**
    - Malaria *Salmonella* Anaemia Neutrophil Bacteraemia

7. **Establishing Model for Zika Infection**
    - Zika Virus, Pathology, Immunity

8. **Explore the mechanisms of Persistence of Tertiary Lymphoid Organs (TLOs) and the relationship with secondary lymphoid organs**
    - Tertiary lymphoid structures, salivary glands, immune response, inflammation

9. **Inflammatory responses to infection and insult**
    - Inflammation, Viral infection, Immunology

10. **Regulation of immune responses**
    - Immunity, cell development

11. **Bone Marrow Transplantation: Biology and Therapy**
    - GVHD, GVT, Tumour, Leukaemia

12. **Identifying new malaria intervention strategies**
    - Malaria, Plasmodium, genetically modified parasite

13. **Mammalian gene control mechanisms and disease**
    - Gene regulation, chromatin, triplet repeat disorders, position effect variegation, epigenetics

14. **yô T cells and Body Surface Immunity**
    - yö T-cell immune challenge

15. **Immunological memory in transgenic mice**
    - T lymphocyte, virus, tumour, immunity

16. **Immune regulation, metabolism and tissue integrity**
    - T cells; environment, autoimmunity, mucosal immunity, metabolism

17. **ZBTB proteins in lymphocyte development**
    - Lymphoma

18. **Immune cell regulation of epithelial damage and repair**
    - Epithelial, Repair, T cells, auto-inflammation

19. **Molecular mechanisms of T cell mediated immune responses**
    - Immune response, autoimmunity, Egr, viral infection, tumour

20. **Lymphocyte development and antibody repertoire formation**
    - Fighting infection, Antibodies, Ageing, Immune response, White blood cells

21. **DNA double-strand break repair, immunity & cancer**
    - DNA repair, cancer, immune system

22. **Interactions between mast cells and helminths in inflammatory disease**
    - mast cells, helminths, diabetes, arthritis, cardiovascular disease

23. **Complement properdin in immunity and inflammation**
    - Properdin, tumour, diet, stimulation

24. **Inflammation, cell death and cancer**
    - Inflammation, immunity, cancer

25. **Translation of the immunological synapse**
    - Immunological synapse, Tolerogenic DCs, Central tolerance, Atopic dermatitis, Original antigenic sin

26. **Immune activation in health and disease**
    - Immunity, adjuvants, vaccines, allergy, hypersensitivity

27. **Mouse models of chronic inflammatory diseases**
    - Cancer, infections, autoimmune disease, pain

28. **Tregs in lymphopaenia associated autoimmunity**
    - Lymphopaenia, Autoimmunity, Immunotherapy

Project 1	**Immunity against bacterial and paediatric diseases**	
Key Words (max. 5 words)	Vaccines, infectious diseases, paediatrics, bacteria	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our research aims to develop and evaluate vaccines against human pathogens, particularly affecting the paediatric population and developing countries, for which there is currently no vaccines or vaccines that have insufficient efficacy or not adapted to certain countries, and (2) Investigate the mechanisms underlying successful vaccines in an attempt to generate novel and more efficient vaccine strategies. The clinical needs to be addressed are that bacterial infections are a leading cause of death and disease worldwide, dramatically affecting children. Vaccines represent the best hope for prevention. There are no satisfactory vaccines against either *N*. *meningitidis* capsular group B (Meningitis B), the leading cause of meningitis in many countries, *S*. *aureus,* which also affects children but is additionally a major cause of hospital-acquired infections, representing a serious threat to public health due to the appearance of antibiotic resistant strains (MRSA), enteric salmonella bacteria and soil-transmitted internal parasites (worms), which are the most prevalent tropical diseases*.* Respiratory syncytial virus is responsible for severe bronchiolitis in infants, even in developed countries, where it is the main cause of	

	hospitalisation of infants in winter. Our project plan is to: (1) Make vaccines against these diseases (2) Determine whether our vaccines generate immune responses (e.g. antibodies) (3) Determine whether they can protect from disease challenge (4) Determine the safety of the vaccines in several critical conditions (for example in chronic carriers of normally harmless bacteria) (5) Determine the immune mechanisms behind successful vaccines.
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)?	The potential benefits for the 5-year duration of this project are to: - discover new vaccines or vaccine components against the following infectious diseases (meningococcus, *S. aureus*, enteric fever, respiratory syncytial virus, helminths and antibiotic resistant bacteria. We aim to develop and investigate several vaccine candidates for each of the diseases. - Establish the proof of concept and the mechanism of protection induced by these vaccine candidates - Provide sufficient data to support their progression to clinical trial - Compare and identify different mechanisms by which vaccines induce the desired immune responses - Confirm the impact of factors such as genes, identified during clinical studies, in the vaccine-induced responses and side effects. The long term aim is to develop vaccines that will in the future be included in worldwide human vaccination programs. The diseases we are targeting affect primarily babies and young children, and particularly vulnerable populations in developing countries that can benefit most from vaccination. Our ultimate objective is to prevent patients suffering and dying of these diseases. We expect to discover new vaccines and regimens that will be safe and protect humans from a number of major diseases. Our discoveries, if successful will be tested in human clinical trials and could be included in vaccination programs. In addition, a major expected benefit is the

	new knowledge that we aim to bring not only into the vaccinology field, but also in the immunology of each of these disease, through publication of our research. In addition, our program of work with new vaccines and delivery methods, such as needle-free parenteral and mucosal delivery, and new technologies such as understanding the genes involved in successful vaccine-induced immune responses may lead to novel knowledge that will significantly and lastingly improve vaccine development programs, efficacy, uptake and safety.
What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 15,200 mice will be used during the 5 years of the project
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 project will use mouse models to evaluate the immunogenicity of vaccines, and also models of human diseases to evaluate if the vaccine can efficiently protect against the diseases. For the immunogenicity studies, mice receive the vaccines by injection, and blood samples are taken to evaluate the response. The adverse effects expected are mild, these procedures are similar to what would be performed to a human or a baby, except that mice are sedated during the injection to avoid the stress or pain from the manipulation and the injection. Blood samples are performed without sedation, as these are so quick that sedation and short drowsiness would likely induce more stress. The diseases are induced through experimentally exposing the mice to the bacteria or virus. The injections are performed under anaesthetic to avoid pain. Within 2 to 3 days, mice may become ill and show signs of discomfort (less mobile, loss of body weight). Every effort will be made to reduce the welfare cost to these animals by the most refined husbandry methods and providing mashed up food and water on the floor. Mice will be monitored daily at the peak of infection and not allowed to suffer moderate discomfort for more than 48 hours. Then they will either recover, or be immediately killed to avoid suffering. The models of infections are not expected to cause severe pain because the experiments will be stopped before mice become sick, as the effect of the vaccine can be observed by measuring the amount of bacteria or virus in the body

	before it becomes too high. Every effort will be made to ensure protocols are continuously refined – in particular by identifying challenge doses and routes of administration that cause reduced animal suffering and distress, and by identifying early timepoints after challenge that allow the evaluation of the vaccine effect without letting mice become ill. Control measures and humane endpoints are used so that any adverse effects experienced by animals are moderately severe at the maximum. All animals will be humanely killed at the end of a specific set of procedures. They will not be kept alive and re-used for other experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We do not use animals in our vaccine production, however, we have to test the vaccines initially in animals before we can trial them in humans to ensure they are safe and efficacious. Responses to vaccines are complex and at present there is no other way of testing them than using animals, there is no non-animal system that recapitulates the function of the immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We calculate the minimum number of animals needed to tell us whether the vaccines work, using statistical methods. We also combine experiments so that controls do not need repeating. Vaccines are quality-controlled using laboratory techniques, therefore, only vaccines that are of the expected quality are investigated in animals.
3. 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 use mice because they are the less sentient specie studied, with an immune system that is well characterized, and there is an understanding of how responses in mice may translate into what we would find in humans.There are extensive sets of reagents available for analysing vaccine-induced immune responses in mice. The project also involves genetically altered animals (GAA) in order to investigate the role of specific genes in the immune response, and to identify which part of the immune system provides the immune responses and protection. We are minimizing animal suffering during administration of substances, by using the most refined route, and where possible needle-free injections that we are developping. We use short

	anaesthesia, respect maximum volumes indicated for each route, and experiments are only performed by highly trained professionals. Animals are exposed to infectious agents, we use the mimimum dose required to induce an infection course that is well characterized and thus animals can be controlled, and time frame reduced in order to reduce clinical signs. Most of experiments are stopped before animals get ill, because we can count the infectious agent in the body, therefore the clinical signs are not needed as an experiment readout. When possible, asymptomatic colonization rather than injection is used, and to this end contaminating the cage is sufficient rather than manipulating each animal. We use serial blood sampling to identify early markers and thus reduce the time frame of experiments, and use non-invasive procedures to monitor clinical signs.

Project 2	**Poxvirus virulence and immunogenicity**	
Key Words (max. 5 words)	Immunisation, vaccines, immunology, virus virulence, protein function	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objectives of this project are to: 1) understand how individual proteins made by vaccinia virus function during infection, particularly how they influence the ability of the virus to cause disease and how they affect the immune response to infection 2) study how the immune system works, particularly how alterations in the type of immediate response to infection (innate response) can influence the longer term development of immunological memory (adaptive response) to provide protection against re-infection 3) develop vaccinia virus as a more effective and safer vaccine 4) learn how different treatments can provide protection against infection by poxviruses	
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)?	**Potential benefit.** The information gained will increase our understanding of how viruses evade the immune response to infection and cause disease. It will also advance our understanding of how the immune system works, and will enable the design of safer and more effective poxvirus vaccines for use	

	against infectious diseases and cancer. **Likelihood of achievement.** The work is very likely to be successful because the methods we will use to answer our scientific questions are well developed and we possess the necessary expertise and peer-reviewed funding from several agencies to support delivery of this work.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice are the only species that will be used over the 5 years of this project and it is estimated that up to 10,750 mice may be used.
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 work proposed in this project is to infect mice with viruses by either the skin (in the ear) or the nose and then study the outcome and the immune responses induced. Infection via the nose will cause a chest infection that results in gradual weight loss of up to 20% and other general signs of illness (such as ruffled fur and arched backs) before the animals recover. This has an overall severe band, although it is anticipated that less than 50% of the animals will reach this level. Infection via the skin (earlobe) may cause a local lesion of up to 5 mm that does not spread and the animals show no general signs of illness. The lesions heal within 3 weeks. Some animals may have a small hole in the ear (1-2 mm) after healing. Overall this has a moderate severity band. The breeding of mice will have either no adverse effects or only mild effects (so a mild severity). Lastly, the immune suppression of mice by irradiation will give a transient weight loss (up to 10%) before recovery as the immune system recovers following transfer of immunological cells to these mice. These mice may then be infected with viruses in the skin with outcomes as above. This has a moderate severity band. At the end of all experiments all animals are killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Animals are used because it is not possible to measure virus virulence (the ability of a virus to cause disease) by any other method. Cell culture systems for virus growth cannot measure virulence and nor

use non-animal alternatives	can mathematical models. Similarly, for measuring how effective a virus is as a vaccine, a whole living animal with an immune system is needed, and no surrogate systems can reproduce this.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have extensive experience with the methods and models used. This has shown that groups of 5 animals generally give statistically significant data, but since the contribution of any protein to virus virulence cannot be known in advance, the animal group sizes are not certain and must be monitored (use of pilot experiments with 2 mice / group only). For instance, when the removal of a specific gene gives only a mild alteration in virulence, it may be necessary to use larger group sizes to demonstrate whether or not this difference is significant. Conversely, where deletion of a virus gene results in greater attenuation, smaller groups of animals are needed to establish a significant difference. The same principle applies when measuring the immune response following infection with different viruses. So throughout the project the group sizes will be monitored so that we can use the minimum number of animals to achieve scientifically valid data. In each experiment we use wild type virus, a mutant virus lacking a specific gene and an additional control virus (called a revertant virus) in which the gene has been re-inserted into the mutant virus. In addition, an uninfected control group is included. So if we were to measure the virulence or vaccine potency of a virus lacking a particular gene we would need a total of 20 mice per experiment.
3. 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.	Vaccinia virus is an enigma of virology, for although it is the only vaccine to have been used to eradicate a human disease (smallpox) its origin and natural host are unknown. Therefore the correct species to use when working with vaccinia virus is uncertain. However, it is established that vaccinia virus can replicate effectively in mice and this host has been used widely for evaluation of virus virulence and the potency of this virus as a vaccine and so the inbred laboratory mouse is the chosen model for our work. Although other (large animal) models have been used for evaluation of the virulence or vaccine potency of some other orthopoxviruses, we believe that the inbred laboratory mouse is better than most because the model is simple and gives reproducible results and there are many immunological reagents

	available to make detailed analysis straightforward. Work with genetically altered animals is included so that we can investigate the importance of specific proteins of the immune system in combating infection by poxviruses or other viruses. This will also enable us to explore the function of specific vaccinia virus proteins in immune evasion and correlate this with our other work with these proteins that does not involve animals. Animal suffering will be minimised by, using the lowest dose of virus to achieve objectives, by regular monitoring, and by provision of mashed moist food in some protocols, and by using early humane endpoints.

Project 3	**Development and function of the immune system**	
Key Words (max. 5 words)	Lymphocyte, Infection, Autoimmunity, Signalling	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The immune system plays a crucial role in protecting us from infection by a large variety of micro-organisms, including bacteria, viruses and fungi. White blood cells form a key part of the immune system, and are at the vanguard of fighting infections. Some white blood cells make antibodies, others eat up the pathogens, and yet others provide help to other parts of the immune system, acting as managers. While the immune system is clearly very important for our health, it also has a dark side. Inappropriate or overexuberant activity of the immune system can lead to autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and asthma. Thus it is important that the immune system mount the right kind of a response at the right time. These decisions are controlled by biochemical processes operating inside the cells, and are poorly understood. We will study these pathways in order to gain a better understanding of how the cells make decisions whether or not to mount immune responses.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Better understanding of the biochemical processes that control the decisions made by white blood cells regarding when and where to mount immune responses will lay the foundations of knowledge that will be used by others to design rational therapeutic interventions aimed at either boosting the immune	

project)?	system to mount better responses, or at dampening responses, for example in the context of autoimmunity.
What species and approximate numbers of animals do you expect to use over what period of time?	75000 mice over 5 years
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 large majority of mice to be used in this project will experience little or no adverse effects, and in most cases the adverse effects will be minor and transient. A very small minority of animals will be used to model autoimmune conditions such as multiple sclerosis, and these will experience stronger symptoms. All mice to be used in the project will be killed by humane methods.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will generate as much data as possible using in vitro systems. However ultimately, the immune system is a complex tissue dispersed around the whole body, in which many different cell types interact with each other. The function of the immune system is critically dependent on these interactions, and currently these cannot be replicated in the lab petri dish. Thus the only way to study the immune system is to do so in an animal. Nonetheless as new lab-based approaches become available we will adopt these as soon as practical.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The efficiency of animal usage will be maximised by careful control of mouse breeding programs, generating only those mice that are needed for the studies. Most of the work will use genetically altered mice. The breeding programs will be set up in such a way that they generate both the mutant mice and the control mice at the same time, thereby maximising efficiency. Furthermore, from each experimental animal we will use as many different tissues as possible, thereby minimising the numbers to be used.
3. 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	The mouse is the best choice for these studies for a number of reasons. The technology to genetically alter the mouse is much better developed than that for any other mammal. The immune system of the mouse is very similar to that in humans, and thus serves as a very good model for the human. Finally, the immune system of the mouse has been extensively studied by 1000s of labs around the world

(harms) to the animals.	for 30 years or more, resulting in a wealth of reagents and methods. There is nothing available on a similar scale in any other vertebrate. For most mice in this project they will only undergo one regulated procedure – they will be bred to generate genetically altered offspring, which for the vast majority will generate no adverse effects. These mice will then be killed by a humane method and their immune system analysed in the lab. Only a small minority of mice will undergo further experimental procedures, for example being immunised or being bled. Again the large majority of these procedures are expected to produce no adverse effects, or only minor transient effects. For example, in order to minimise welfare costs to the mice, they will be immunised with microorganisms that do not cause harmful symptoms to develop.

Project 4	**Genetic and physical control of B cell activation**	
Key Words (max. 5 words)	Antibody responses, activation of immune cells, signalling, endocytosis	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Antibody responses can provide effective protection against a wide range of infections. Production of antibodies is regulated by communication between immune cells as they contact each other in the body. Erroneous cellular contacts lead to poor immune responses, autoimmunity or development of cancers. We aim to understand the genetic and physical factors that control contacts of B lymphocytes with other immune cells during antibody responses. We are identifying genes and proteins that generate cellular forces that allow B cells to mechanically detect and internalise pathogens. We are also developing new techniques to measure and manipulate these forces by nanomechanical methods. The ultimate goal our studies is to improve mechanical properties of vaccines for better B cell stimulation and production of highly effective antibodies.	
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)?	This research will provide important new insights into the basic mechanisms that regulate protective antibody responses and into the pathology induced by abnormal activation of B cells. Knowledge generated by this research can aid the development of new vaccination strategies to improve antibody-mediated protection against infectious diseases, and	

	can provide novel targets to eliminate pathological B cells, for example during autoimmune reactions or upon B cell malignant transformation.
What species and approximate numbers of animals do you expect to use over what period of time?	Species: Mouse Period: 5 years Animal number: 6000
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?	Most of our procedures are expected to be mild with no more than 10% of the animals to reach moderate severity. However, it is not possible to fully predict the nature or severity of any potential defect generated during the course of the studies. We will set humane endpoints that allow molecular analysis of the B cell response before development of adverse effects. All mice will be carefully monitored and animals exhibiting any unexpected harmful phenotypes will be killed using a Schedule 1 method. Deletion of genes in early B cell development and bone marrow transplantation may result in immune deficiency, the effects of which will be controlled by keeping the mice in a barrier environment and application of antibiotics. Our protocols, including immune challenge, have been optimised to induce only transient discomfort. Animals will be monitored for distress and killed by a Schedule 1 method if they appear hunched, immobile after touch or display other non-transient moderate clinical signs such as weight loss, reduction in body temperature or lack of normal movement.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The reason for using animal models for our experiments is that the development of the cells of the immune system and their complex interactions cannot be fully recapitulated outside of the animal. In addition, disease conditions associated with immune system pathology develop as a result of imbalance between factors that are impossible to reconstruct in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are working within an institute where sharing and carefully organised breeding reduces the overall numbers of animals used. In addition, our experiments use robust determination of the minimal possible numbers of animals to use in experiments. We have also pioneered modern single-cell analysis techniques that dramatically reduce the number of animals without compromising the quality of the

	experiments.
3. 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.	Mouse immune system, including antibody responses, has been extensively studied and closely resembles the human immune system. An enormous amount of reagents is available for studying the immune system of a mouse, and mouse transgenic and knockout techniques are well established and mutant strains are widely available. To our knowledge the laboratory mouse is the least sentient species that fulfils the requirements of this research. Most of our procedures, including breeding, and immune challenge are expected to be of mild phenotype with not more than 10% reaching to moderate signs. All mice will be carefully monitored for possible harmful effects and if necessary killed using a Schedule 1 method. Immune deficiency due to genetic defects or irradiation will be controlled by specific pathogen free conditions and by supplementation of drinking water with antibiotics. Animals challenged with model antigens are expected to exhibit only transient discomfort and no lasting harm. All dosages of treatments and infections will always be targeted to achieve a suboptimal response with mild signs in control animals. Animals showing more than 20% weight loss, loss of normal movement, a hunched appearance, or signs of pain (such as grimacing), will be killed by a Schedule 1 method.

Project 5	**Immunity to ruminant endo- and ecto-parasites**	
Key Words (max. 5 words)	Vaccine Parasite Sheep Cattle	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
	√	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Infection of humans and livestock with parasites can have devastating effects on health and production, affecting food security in developed and developing regions. Currently, these pathogens are controlled using drugs and pesticides; however, populations of the pathogens with multi-drug resistance are now relatively commonplace. Despite decades of research, the development of vaccines against parasites has been largely unsuccessful. However, successful prototype vaccines have recently been developed for use in sheep and cattle to control parasitic gastroenteritis (PGE), caused by infection of the gut with parasitic nematodes (worms) and sheep scab, caused by parasitic mites, which are two of the five most important diseases for livestock farmers from both a financial and a welfare perspective. The specific objectives of the project are therefore: 1) To understand the nature of the protective immune response which is induced by administration of these vaccines 2) To use this information to improve the effectiveness of the vaccines such that they will provide an important tool in the control of production and welfare-limiting parasitic diseases of livestock.	

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)?	The benefits that could derive from the project are clear: The vaccines that are being developed in the project will directly benefit livestock by protecting them from parasitic disease. This benefits both the welfare and productivity of the vaccinated animal but also reduces reliance on synthetic chemical, adding to global food security and reducing environmental contamination. In addition, the parasites which are being investigated here have close relatives which parasitise humans. The large-scale programmes which exist to produce vaccines against parasites of humans (e.g. the human hookworm vaccine initiative) may also benefit from the vaccine development and discovery work in this project.
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep and cattle. 830 sheep, 190 cattle over 5 years.
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 adverse effects on the animals will be of moderate severity and will be associated with the effects of the parasites on the host. In practice this is likely to involve the formation of an itchy scab on the skin during sheep scab infestation. Infection with parasitic nematodes is likely to cause less pathology but may cause some inappetance and/or diarrhoea.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Neither scab mites nor parasitic nematodes of sheep and cattle can be maintained off-host or in alternative animal species so there are therefore no alternatives for the use of sheep and cattle in these studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All animal studies are planned in consultation with statisticians prior to submission to the local ethics committee in order to provide adequate group sizes for the most appropriate statistically robust analyses while minimising the number of experimental animals. Prior work using these infection models has established the optimal group size for vaccine trials, although each experiment is discussed with statisticians and these group sizes may be reduced should new data become available.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	The species being used, sheep and cattle, are the natural hosts for the parasites being tested and are also the target species for the vaccines being developed, so are the most appropriate species to be employed here. Vaccination and challenge of sheep

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	consists of 3 immunisations with the candidate antigen(s) in adjuvant, two weeks apart and animals are then experimentally challenged with either a “trickle infection” of infective worm larvae to minimise pathogenesis or with a mite infestation which will be carefully controlled. During infection and vaccine testing, sheep are routinely monitored by veterinary staff and are treated with veterinary medicines if required, based on clinical symptoms.

Project 6	**Salmonella pathogenesis during asymptomatic Malaria**	
Key Words (max. 5 words)	Malaria *Salmonella* Anaemia Neutrophil Bacteraemia	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	**x**	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to understand why people infected with malaria are at high risk of developing invasive bacterial disease, especially non-typhoid *Salmonella* infections. Co-infection with malaria and *Salmonella* carries a very high risk of death. In particular, we will test the hypothesis that very low levels of malaria infection (so-called asymptomatic infections, which are highly prevalent in endemic areas) increase the risk of severe bacterial infections and, if so, whether treatment of these chronic, low levels of malaria infection will reduce the risk of developing severe bacterial disease. We also wish to better understand the cellular mechanisms underlying this detrimental interaction between malaria and *Salmonella*.	
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)?	*Salmonella*/malaria co-infections are understudied, even though they represent a major cause of mortality in sub-Saharan Africa. We expect that our research will provide important and novel insights into specific immune defects that explain increased susceptibility to systemic *Salmonella* infection and may provide the rationale for clinical trials of anti-malarial treatment to reduce the incidence of severe bacterial infections in malaria endemic populations. Further, the results of our studies are likely to provide	

	novel paradigms of how polymicrobial infections affect disease outcome.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse only with a maximum of 1250 animals per year.
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?	Malaria and haemolytic anaemia increase the severity of subsequent Salmonella infections. Co-infected mice are expected to develop high bacterial loads and signs of systemic sepsis. All experimental animals will be culled as soon as they reach their humane endpoint.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Due to the complex nature of the immune system in mammals it is impossible to achieve the features of an immune response outside of a complete animal model. In order to fully assess the interaction of two unique pathogens, it is necessary to conduct the experiments in animals in order to understand mechanisms of disease in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise group sizes by reducing sources of variability (inbred strains of mice, age matched, housed in individually ventilated cages). Pilot studies will define the minimum number of mice per group required to give robust, repeatable and statistically significant results. Statistical advice will be sought where necessary, for example where multiple outcomes are possible, or where a complex design is required.
3. 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.	Mice are the biological system of choice. They are sufficiently immunologically similar to humans to allow confident extrapolation of findings, yet they are considered neurophysiolgically less sensitive than dogs, cats or non-human primates. Moreover, the size, social structure and husbandry requirements of mice are conducive to humane care in pathogen containment settings. All staff will undertake regular refresher training in animal handling and experimental techniques, and skills sharing between researchers will be encouraged. Detailed literature reviews will be undertaken prior to the introduction of any new biologic or technique to ensure best practice.

Project 7	**Establishing Model for Zika Infection**	
Key Words (max. 5 words)	Zika Virus, Pathology, Immunity	
Expected duration of the project (yrs)	2	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We are looking to develop an animal model for Zika that will enable the world to quickly develop medicines and public health measures that prevent or control the devastating effect of this virus in South and Central America during pregnancy and the neuropathology suffered by a proportion of adults after infection. The events occurring in Brazil and elsewhere were unexpected compared with the course of infection in Africa and Asia where infection is well established. We do not know whether it is a result of the virus in South America having mutated or because large numbers of adults have not developed immunity because they were not exposed to the virus as a child.	
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)?	It is hoped that the work will identify where the virus goes to in the body after infection and the responses that are made by an infected individual. A particular interest is where the virus goes in the brain and other nervous tissues because the diseases it causes are associated with damage to nervous tissue. This information will determine whether effective treatments can be developed. In addition, it is hoped that this work will determine whether exposure to the virus generates immunity that controls subsequent infections. This is critical information if the world	

	wants to develop an effective vaccine.
What species and approximate numbers of animals do you expect to use over what period of time?	The studies will focus on developing monkey models either in macaque monkeys, marmosets or tamarins. It is envisaged that the work will require up to about 120 monkeys in total. Studies will usually last less than 42 days for most studies looking at the distribution of virus in the brain and other tissues and up to 90 days for studies designed to understand the basis of vaccine protection.
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?	In this pilot project, it is not known with certainty what side effects may be in each species following infection with Zika. The virus is reported to cause mild fever in macaque monkeys and very recently reported to be detected in tamarins from South America, but no information on any disease symptoms associated with infection have yet reported. In order to inoculate virus and collect blood will require animals to be sedated and may cause moderate adverse events. We also intend to collect cerebro-spinal fluid to sample virus in the brain. This may cause temporary adverse events. We have close links with clinical experts who regularly collect cerebro-spinal fluid and most adverse events, likened to headaches are effectively treated with analgesics. At the end of all protocols animals will be killed humanely allowing extensive post mortem to determine where the virus is located and what effects the virus has on the host. In the monkey species where infection occurs and the infections mirrors the situation described in humans, then additional studies will be performed. Monkeys will be infected during the early stages of pregnancy and the effect of the virus on the mother's reproductive tract and on the development of the foetus will be determined. These studies will last only up to 6 weeks and terminated before the foetus is viable outside the mother.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This study has to use animals because we need to look at the impact of Zika on the whole individual in order to understand why virus infection in South America is different from infection in Africa.
2. Reduction	As a pilot project all initial studies will be performed in pairs of animals to establish the principle whether it is

Explain how you will assure the use of minimum numbers of animals	worth pursuing further studies in that species. The major goal is to establish infection of which species most faithfully replicates the infection and disease that has been reported in humans in South America.
3. 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 proposed studies must be performed in non-human primates because these species have anatomical and physiological features most similar to that of humans in the tissues and organs of particular interest i.e. the brain and nervous system and the female reproductive system. The experience of the group in handling these species will ensure that any deviation from normal behaviour following infection with the virus will be detected very quickly and treatments to deal with symptoms initiated.

Project 8	**Explore the mechanisms of Persistence of Tertiary Lymphoid Organs (TLOs) and the relationship with secondary lymphoid organs**
Key Words	Tertiary lymphoid structures, salivary glands, immune response, inflammation
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:

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

Have you ever woken up with swollen glands in your neck? These glands, called lymph nodes, are structures that house stromal cells which provide nourishment and survival signals to the infection-fighting cells of the immune system. Once the infection is cleared, the lymph nodes return to their normal size by drainage by specialised stromal cells named lymphatic vessels. In chronic autoimmune diseases like Sjögren's syndrome this is not the case. Infection-fighting cells become out of control and take up residence in other organs like the joints or the salivary glands instead of the lymph nodes. These cells organise into tertiary lymphoid organs (TLOs) and are often associated with poor outcome of disease and contribute to lymphoid cancer development. Are stromal cells involved in the persistence of those TLOs? Or is inflamed tissue not able to return to homeostasis due to defective drainage of infection-fighting cells? By answering these two questions we hope to better understand the potential role of TLOs in chronicity

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)?

The detection of TLOs in the target organs of autoimmune disease changes disease prognosis. Their presence might herald the later development of cancer. We provide patients with this information by performing routine tissue biopsies, however, we cannot really answer the simplest question: why are these structures there and how do they lead to the development of worst disease and sometimes cancer? Understanding why TLOs persist in the tissue in autoimmune diseases such as Sjögren's syndrome is an area of significant unmet need and has to be solved if we are to effectively target TLO associated diseases and their comorbidities. Moreover, due to the structural and functional similarities between secondary and tertiary lymphoid organs we need to understand whether TLOs can be safely treated

therapeutically without interfering with the physiological function of the secondary lymphoid organs (such as lymph node and spleen).

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

Over 5 years, we would expect to use no more than mice in total 13,000 animals for scientific protocols and 20,000 to breed the genetically altered strains required

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?

Breeding of genetically modified animals – we expect adverse effects of mild severity such as minor weight loss. If pain is observed, as evidenced by tip-toe walking, animals will be humanely culled however, this is not expected. Induction of conditional genetic modification – we expect adverse effects due to tamoxifen or diphtheria administration of a moderate severity such as weight loss of up to 20% and reduced activity. This is reversed upon cessation of tamoxifen treatment with little-to-no lasting effects once genetic deletion has been achieved. The cannulation, immunization and splenectomy models have been refined to minimize the side effects related to the procedure and to the use of anaesthetic. Where animals will show signs of distress and lack of recovery post anaesthesia advice will be seek from the personnel. Mice might be re-treated with recovery agents and if recovery is still not satisfactory mice will be culled by schedule 1.

Application of the 3Rs

Replacement

This program of work is aimed to address complex organ functions such as the generation of the immune response within lymphocyte aggregates at peripheral (ectopic) sites and the independency of these structure from the classical secondary lymphoid organs (lymph nodes and spleen).

Whilst we have spent long time devising a range of in vitro co-culture models that have furthered our understanding of leukocyte-stromal cell interaction in chronic inflammation we believe that the questions we aim to address in the work cannot be resolved in simple co-colture systems

Reduction

Statistical analysis will ensure that we use the minimum number of mice per group that will be informative will be performed.

Inducible global knockouts will be used in adult mice prior to any cell-specific genetically altered mice. This will ensure that we firstly identify a gene which shows an effect following our protocols, before generating multiple cell-specific transgenic strains. This will also minimise developmental defects in cell specific knockouts that could compromise the results.

We are using a staged approach, involving pilot studies to ensure that an appropriate number of animals are to be used. To maximise the information gained from a single animal we aim to take perform multiple *ex vivo* analyses on each individual. Animals in all protocols will be humanely killed with or without having blood removed from a major vessel or the heart which will only be performed under terminal anaesthesia

Refinement

Inducible transgenic strains will be activated by the most refined interventions possible to minimise stress and pain. The procedures listed have been optimized to minimize the discomfort for the animals. Analgesia is administered to all mice prior to anaesthesia to reduce the discomfort in the post-operative phase. We now immunize mice by single subcutaneous injection in the upper surface of the paw and not in the foot pad which causes less discomfort. Mice that undergo procedures and/or mice with uncharacterised genetic mutations will be monitored closely and appropriate action taken if they are deemed to be suffering. Animals will be humanely culled unless, in the opinion of the NVS or NACWO, suffering can be remedied promptly and successfully using no more than minor interventions, such as pain relief and hydration

Project 9	**Inflammatory responses to infection and insult**
Key Words	Inflammation, Viral infection, Immunology
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;

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

Mosquitoes can pass disease to humans when they bite, which includes infections caused by viruses. Most such infections are usually found in the tropics, but a changing climate and globalisation means their range has spread at an alarming rate. Europe, once confident in its isolation from substantial epidemics is now at risk, as witnessed by the recent spread of viruses such as chikungunya. Therefore it's important we understand how these viruses cause disease, so that we can develop treatments and better predict how future outbreaks unfold. This project will seek to understand the complex biological interaction between biting mosquitoes, the viruses that they carry and their mammalian hosts. We have evidence that mosquito bite inflammation is highly counterproductive and helps viruses establish infection in the skin. This project will work out how the immune system responds to mosquito bites and how viruses spread from bite sites to the blood and other tissues. This will be useful to scientists, health professionals, drug companies and policy makers who shape our response to these epidemics.

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)?

These studies are of central relevance to a better understanding of infectious and inflammatory diseases. Our proposed work provides numerous opportunities for finding novel and important targets for the development of new drugs. We have two main aims; 1) to see if we can treat the virus infection at mosquito bites to prevent disease 2) to determine how these viruses spread around the body, which will be important in the design of new vaccines and medicines.

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

Our studies exclusively use mice and we anticipate using about 5,300 mice over the 5-year timeframe of this project. Note that 1000 mice from protocol 1 will be transferred (in continuous use) in protocols 2-7, so that the total mice used will not exceed 5,300. 500 of the mice in protocol 1 will be used as breeders to maintain transgenic lines.

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?

At the end of all procedures under this license, animals will be humanely killed. The majority of procedures to be carried out are associated with a 'mild' or 'moderate' severity rating. However, we are proposing to use one procedure associated with a 'severe' severity rating. Mice on this procedure will be monitored carefully and treated, on advice from local veterinary surgeons, in a way that minimises distress and suffering. Once they reach a severe disease rating they will be immediately culled to prevent suffering.

Application of the 3Rs

Replacement

The immune and inflammatory responses are complicated involving numerous different cell types and molecules. These are carefully orchestrated in an intact animal in ways that cannot be recapitulated using non-animal alternatives.

Reduction

We have over ten years experience of working with animal experimentation and have developed robust protocols involving the minimum use of animals required to provide statistically significant analysis. We also obtain advice from statistical analysis colleagues regarding the design of new experiments.

Refinement

The mouse is the species of choice and it can be genetically manipulated to alter gene function in ways that are not currently possible using other mammalian species. In addition numerous reagents are available for examining, and intervening in, immune and inflammatory responses in mouse models.

To minimise harm to animals, especially those on procedures with which we have less experience, animals will be monitored regularly for routine signs of ill health or distress. Anaesthetics will be used as appropriate to the procedure being undertaken and advice from local veterinary surgeons will be sought in any situation where animals are showing unpredictable signs of ill health or suffering. Occasionally we will insert ‘osmotic pumps’ under the skin of mice, so that drugs can be given in a more effective and less invasive manner. Because this involves minor surgery, analgesics will be used as appropriate under advise from the NVS. In all cases, if animal suffering is obvious or sustained, mice will be immediately removed from the study and culled to prevent further suffering.

Project 10	**Regulation of immune responses**	
Key Words (max. 5 words)	Immunity, cell development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project investigates how antibody responses are generated. Tissue architecture is an important principle that helps to optimize and control immune responses. B cells are highly migratory and constantly move between defined areas within the tissue in order to receive information in a timely manner. However, the manner by which B cells are attracted to the right place at the right time and the interactions they form with other cells during an immune response are still largely unknown. We aim to understand the principles of these processes in order to understand how protective humoral immunity is obtained in response to vaccination and how dysregulation of these processes can lead to immunological disorders.	
What are the potential benefits likely to derive from this project (how science could be	Our studies aim to improve our basic understanding of how humoral immune responses are regulated. Insights gained from these studies will have important	

advanced or humans or animals could benefit from the project)?	implications for our ability to develop novel vaccines that will induce better antibodies with long-term immunological memory. In addition, these studies may also promote our understanding of how dysregulated antibody response develops, knowledge that may help to identify new strategies to treat immunological disorders (e.g. autoimmune diseases, allergy, chronic inflammation).
What species and approximate numbers of animals do you expect to use over what period of time?	This project license requires the use of mice. In addition to wild type animals, we will also crossbreed different types of genetically modified animals, in order to generate animals with defined immune system molecular defects. Crossing different strains and breeding them will require ~20,000 mice over a period of 5 years.
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 maximum severity of the protocols in this project is 'moderate'. Adverse effects under this licence may include discomfort due to procedure, weight loss, moderate pain or irritation due to inflammation. If during the course of any of the techniques or the experimental period an animal exhibits deviation from the normal health, as assessed by food and water intake, social behaviour and general appearance, the animal will be killed using a Schedule 1 method. All the animals involved in the procedures will be killed at the end of the protocol, or before, if stress signs approach the limit of the moderate severity classification.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of our proposal is to understand the molecular mechanisms of this movement and the type of signals the B cells receive in specific locations and time points during an immune response. These studies directly investigate the complex interactions between immune cells and their physiological environment, which cannot be replicated in tissue culture.

	Our studies focus on the regulation of adaptive immune responses, which have evolved in vertebrates, therefore excluding the usage of lower organisms. The laboratory mouse is the species of choice for studying these questions because it shares many similarities with humans as reflected by the similar organization of lymphoid tissues and the significant homology that exists between mice and human with regard to genes and proteins that regulate the processes we are interested to investigate.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The large number of mice required for our studies is largely due to the need to cross many different strains in order to obtain mechanistic understanding of immune responses. We will make every effort to reduce the number of mice used in these studies by using new techniques to create multi-allele mice (e.g. TALENS, Cas9) and freezing embryos and sperm of strains that are not being actively used. We will use appropriate statistics and careful experimental design. We will combine our extensive experience with literature and other sources in order to determine the minimal number of animals needed to obtain a significant result in each experiment. When clear statistical information is not available, we will perform 'pilot' experiments with small numbers of mice. When possible, we will combine experiments to maximize information obtained from each mouse. When possible, we will include intrinsic controls to reduce the numbers of mice required per experiment by 50%. To further reduce number of control groups, we will aim to combine experiments. We will aim to use mice from a similar age and sex group to reduce variation in the data. To reduce number of breeders, we will maintain careful documentation of the number and type of breeders to help organize the colony and ensure no unnecessary breeding is carried out. We will further optimize the breeding strategy to

	generate the correct genotype with as few mice crosses as possible. Embryos of strains that are not currently in use will be frozen. Whenever possible, we will use *in vitro* systems.
3. 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.	A number of approaches will be used throughout the project to minimise adverse effects and suffering to the animals. We will pay careful attention to animal husbandry and provide environmental enrichment and co-housing to avoid social isolation. We will make every effort to reduce the number of procedures per animals and to minimize the discomfort involved during each procedure. This will be achieved through careful experimental design and by ensuring that the researcher performing the procedure is fully competent and understands the protocol and its limitations. We will aim to use the least painful substance and route of administration and make sure that it does not exceed the limits of the allowed amount. When possible, we will use anaesthetics to reduce temporary discomfort during a procedure. When surgery is involved, we will use appropriate aseptic techniques, monitor the animals before during and after the procedure. Special attention will be given to the husbandry of animals after surgery to monitor that they recover well. When mice are subjected to treatments that may cause them moderate pain, a human practice will be exercised to limit the length of procedure to the shortest possible time. In all protocols, we will follow clearly defined action points, monitoring schemes and human end points to minimize suffering of animals.

Project 11	**Bone Marrow Transplantation: Biology and Therapy**	
Key Words (max. 5 words)	GVHD, GVT, Tumour, Leukaemia	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Treatment methods that use one's own immune system to treat cancer have been extremely beneficial in recent years. However, progress has been limited with respect to blood cancers, which include diseases such as leukaemia, which is a debilitating disease that requires complicated treatment procedures. Blood cancer patients are treated with bone marrow transplantation (BMT) that ideally both cures the cancer and provides the individuals with a brand new immune system. In most cases, patients who undergo BMT develop disease conditions such as graft versus host disease (GVHD) while undergoing treatment. GVHD is a debilitating disease that causes significant mortality in patients undergoing BMT. Additionally, some treatments have only limited anti-cancer response (Graft versus tumour effect; GVT). Using mouse models for understanding the biological problems associated with GVHD and GVT and testing novel therapeutics for GVHD and GVT is an extremely important field of	

	research for both children and adults who suffer from blood cancers. The objectives of the current proposal would involve understanding the basic biology of GVHD and GVT in a mouse model of BMT. Mice will be given a treatment schedule that closely mimics that which patients with blood cancer receive. The transplanted mice will then be treated with new medicines that minimize GVHD while maximizing GVT. These studies will help in addressing the dire clinical needs that are currently required for better treatment of blood cancers in children and adults.
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)?	The proposed study will advance the field of bone marrow transplantation in many different ways including the development of cutting edge cancer treatment strategies, which reduce the detrimental effects that the cure (cell transplant) can have on the patient. This study will also understand the basic biology of GVHD, which will result in developing new methods of transplantation that can prevent GVHD but maintain anti-cancer effects for patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mouse as a species for the purpose of this project. We will minimise the number of animals used in any one experiment by using careful statistical analysis and appropriate control treatment groups., Depending on the data generated, trials that show promise may need follow-up studies with greater numbers of animals (typically no more than 20 per group). We predict that we will use 17500 mice in order to obtain meaningful results from our treatment regimens.
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?	Animals will receive bone marrow cells from donor mice that have completely different immune system. This will result in host mice developing a strong immune response that will be treated with medicines that can decrease side effects from giving these transplants The adverse effects that the animals may suffer will be limited due to the experience of the investigator with these animal models. However, a few adverse effects can be predicted. For instance, during the transplantation procedure, the animals will be

	anaesthetized and this may sometimes result in the animals not recovering. In these instances, animals will be humanely killed. To prevent this, warming pads and good surgical procedures will be used. A major complication of GVHD will be weight loss, animals that do lose more than 25% of their body weight will be humanely killed. However, immediately after the transplant, animals will be monitored daily until their weights are stable and fluids will be provided. Another complication of transplantation could be infections. Animals will be treated with antibiotics two days prior to the transplant and will be maintained on antibiotic water for the rest of the period of the experiment (usually 21 days). Since the animals will also be transplanted with a relevant tumour, the size of the tumour will be monitored daily and animals will be humanely killed if the tumour becomes too large. Animals will also be humanely killed if on examination, if the animals were moving slowly due to the tumour. The animals will also be provided with gel meals to combat any dehydration prior to and post transplantation. Animals are expected to show no more than moderate severity and will be humanely killed if deemed to be in danger of exceeding this. At the end of the procedure, after the animals are humanely killed, their organs will be removed to study their response to cancer.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Pilot data has been obtained that suggests that immune therapeutic strategies work in preventing GVHD but also in treating GVT. However, the interaction of the various parts of the immune system cannot be understood from in vitro assays and requires the use of whole animal models prior to be utilized in clinical trials to treat people with cancer. Relevant studies using predictive mouse studies will therefore establish the maximum benefit and efficacy of these agents as novel therapeutics for human cancer and inform the appropriate design of clinical trials in man.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We will utilize the minimum number of animals based on appropriate statistical calculations that will provide maximum information on whether the treatment has an effect in providing anti-tumour responses. Hypotheses and experimental designs will be crafted to minimise the numbers of mice used and the duration of experimental studies.
3. 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.	Emerging literature show that the immune system of both human and mouse may be even more closely related than previously thought. Mouse models of human disease are well accepted in the wider scientific community and many medicines that have shown efficacy in mouse disease models have been successfully used to treat patients in a clinical setting. The research to be conducted in this project will aim to understand the biology of BMT, which is the most complicated immune therapy available for treatment of blood cancers. Mouse model of BMT is hence the most refined for this research project due to the similarities in the immune system while providing a context for immune cell interactions in the living body after BMT. Moreover, the availability of reagents that help study mouse immunology is much more vast as compared to other larger animal models or non-human primates. These benefits will help in the progress of BMT research which will result in the development of better clinical trials for patients with blood cancers. Animal suffering will be minimized by daily monitoring of mice under experimental protocols to detect distress. Experiments will be terminated once the objectives and end points have been collected. Animals will be subjected to anaesthesia prior to invasive procedures and suitable post-anaesthetic care will be provided. Humane endpoints will be applied to minimize suffering in each model used in the study. A summary of endpoints are as follows • Mice will be humanely killed if they lose more than 25% body weight loss from that of initial weight. We will closely follow the clinical scoring sheet to monitor the health and well-being of the animals during the course of the experiments outlined in

	the respective protocols 2-6. • Supportive care will be provided to mice in danger of losing significant body weight as dietery supplements. Animals that look unwell will be monitored twice a day for a week for signs of dehydration, weight loss and piloerection. In case, weight loss cannot be reversed within a reasonable timeframe (eg., 7 days) and reach more than 25% body weight loss from initial weight, the animals will be humanely killed. • Mice will be treated with antibiotics immediately prior to and post BMT • Mice bearing tumours >1.2cm mean diameter will be humanely killed in accordance with published guidelines

Project 12	**Identifying new malaria intervention strategies**	
Key Words (max. 5 words)	malaria, Plasmodium, genetically modified parasite	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to improve our understanding of malaria parasite biology in the vertebrate host and insect vector. The objectives are to identify new molecular targets that can be used to develop novel inhibitors of parasite development and pathogenesis, to be able to better combat malaria in future.	
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)?	This project will contribute to our general understanding of malaria parasite biology and will identify new targets and intervention strategies for parasite control. Promising data can then be taken further in human malaria studies and ultimately this can lead to the development of new antimalarial chemotherapy or vaccines, which will have great benefit for global public health. There are also potential benefits to animal health where the research leads to development of more general antiparasitic drugs. Immediate benefits: identify new targets and intervention strategies for parasite control in mouse malaria models. Mid-term benefits: validate new targets in the human	

	malaria context. Long-term benefits: development and application of new antimalarial measures.
What species and approximate numbers of animals do you expect to use over what period of time?	5,000 mice over a period of 5 yrs.
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?	Infected animals will develop clinical signs of disease that are not expected to exceed moderate severity level, and in most cases are not expected to exceed mild severity level. At the end of experiments animals will be humanely culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Culture of rodent malaria parasites in the presence of suitable host cells is not suitable for propagation of the parasite. Thus, parasite culture does not provide a viable alternative to the use of live animals for parasite maintenance or production, nor for studying parasite biology and pathogenesis in whole animals or for target assessment studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have over 15 years experience in using rodent malaria models in similar research projects to ensure that all experiments have been optimised so that minimal numbers of animals are used to answer our scientific questions and test our hypotheses.
3. 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 the most appropriate and highly defined host for rodent malaria models. Genetically, the mouse is a highly defined host with a complete annotated genome, and many strains (including genetically altered) of mouse are available. Many aspects of rodent malaria biology, in particular transmission, can be easily extrapolated to human malaria species. Procedures will typically be carried out in a way that minimises pain, suffering, distress or lasting harm, while ensuring that the experimental targets can be

	reached. Administration of compounds will typically be carried out via routes of administration, with frequencies of administration, and with volumes administered that cause no more than mild transient pain and discomfort and no lasting harm. Blood withdrawal (sampling) during the course of experiments will typically be carried out by collecting blood from a superficial tail vein that has been punctured with a needle. The frequencies of blood sampling and the volumes collected will be such that no more than mild transient pain and discomfort and no lasting harm is caused. Intervals between procedures will typically be such that the animals have time to recover and no lasting harm is caused.

Project 13	**Mammalian gene control mechanisms and disease**	
Key Words (max. 5 words)	Gene regulation, chromatin, triplet repeat disorders, position effect variegation, epigenetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to understand how cells establish and maintain particular gene expression patterns during development and how dysregulation of genes can lead to disease. Such understanding is crucial to the development of new therapies for incurable diseases in the future.	
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)?	The key biological question we are addressing is how do cells know and remember which genes to keep active and which to silence and how does this vary in disease and between the sexes? Understanding this process will help us to understand diseases caused by failure to regulate genes correctly and will provide important insights into how easy it is to stabilise gene expression patterns or change them. Clearly such knowledge is crucial if we want to intervene in disease processes in the future by modifying gene expression or in stem cell therapy and gene therapy. Important information as to how genes are regulated in mammals and how diseases result when the process goes wrong	

	will lead to more rational and safe ways of treating such diseases in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	We will study approximately 23300 mice over a 5 year period.
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 mice will be bred in a modern and well maintained animal facility. Most mice will be analysed by taking a blood sample or after humane killing. Some will be neutered and may be given hormone replacement therapy to study the effects of sex chromosomes and hormones on how genes are switched on and off. Some mice which develop mild signs of the human neurogenetic diseases will be crossed to modifier mice to see which genes might ameliorate these signs. They will also have investigations similar to those done on humans — brain scanninq and electrophysioloqical studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The process of differentiation of different cell types takes place in a highly coordinated way in developing organisms — we are studying how these changes take place by switching on and off genes and how when this process goes wrong it can lead to disease. Our research promises to find ways to reactivate genes that have been inappropriately switched off in humans and a mouse models that mimic the human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have been able to reduce numbers of mice used by deriving cells from embryos which greatly reduces the numbers of animals required per experiment. In addition the use of novel ways to generate new transgenics promises to reduce the number of animals required.
3. 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	Mice are very well characterised from a biological perspective and the procedures for developing genetically alteration very well established. They display many of the features seen in humans with regard to gene regulation and dysregulation in disease. Mice will be housed in state-of-the-art facilities looked

general measures you will take to minimise welfare costs (harms) to the animals.	after by trained staff and vets with environmental enrichment to ensure well-being. Pain relief will be used where appropriate, and as advised by the veterinary surgeon. Animals will be regularly monitored (more frequently following surgery), and any exhibiting evidence of suffering that is greater than minor and transient and which cannot be addressed by appropriate veterinary treatment will be humanely killed.

Project 14	**yô T cells and Body Surface Immunity**	
Key Words (max. 5 words)	yö T-cell immune challenge	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The core aim of our studies is to understand an important, but very much under-studied aspect of biology: namely the very large numbers of so-called T lymphocytes that sit at body surfaces, e.g. skin and gut, rather than within our lymph glands. We have already shown that these cells can make rapid responses to the dysregulation of their surrounding tissues, and in that light can contribute resistance to cancer. However, to better understand this biology, we need to define the molecules that mediate the interactions between the T cells and their neighbouring epithelial cells and the dynamics of those molecular interactions in health and disease. Such interactions could become biomarkers of tissue- status and/or therapeutic clinical targets, e.g. in inflammatory bowel disease. Thus, as we use the unparalleled scientific power of mouse models to identify new interactions, our clinical research team will promptly investigate the parallels in human clinical materials, in cell culture experiments, and in humanised mice.	

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)?	A better understanding of the interaction between tissues and immune cells in health and in disease will allow for the development of more targeted therapies and more precise biomarkers. We are working towards being able to expand immune cells from patients' tissues, which may be used in therapeutic applications. For this to be possible will rely completely on the fundamental understanding of tissue-associated T cells that we develop in the mouse.
What species and approximate numbers of animals do you expect to use over what period of time?	78000 mice over 5 years
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 large majority of animals in our breeding and monitoring programme will experience little and/or only transient adverse effects, It is possible that the inter-breeding of novel mutant strains will be accompanied by unanticipated adverse effects, such as we found for a very small percentage of mice deficient in butyrophilin-like I (Btnhl) which develop hydrocephaly. Our intensive monitoring of our animals means that any adverse events are detected quickly; animals culled; and alternative breeding strategies and/or experimental methods (e.g. bone marrow transplantation of cells from one genotype to another) are adopted. Where responses to challenges are to be described, e.g. immune surveillance of cancer; capacity to mount immunity to infection; acceptance of graft, the minimum numbers of mice required to demonstrate a clinically relevant effect will be determined by so- called power calculations. All mouse models used will be assessed such that the minimum severity in terms of tumour, infection, allergic or inflammation burden required to show effects will be employed. An example of this is described in our application to continue our studies of cells contributing to inflammation of the nervous system. All mice to be used in the project will be killed by humane methods.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The dynamic responses of body-surface T cells to challenges, such as carcinogens or inflammatory irritants cannot be examined by use solely of dead animals; cannot be adequately represented ex vivo by use of cell lines or mathematical models; and cannot be examined directly in humans where we are severely limited as to what challenges we may make to body surfaces. We therefore require live animal models to accept or to refute hypotheses concerning the molecular basis of tissue-resident T cell biology and their likely pathophysiological importance for human beings. As we learn more about the key interactions between T cells and other cells within the skin, gut, or reproductive tract, so we shall develop organ cultures and heterotypic cell cultures for dissecting those key pathways in more detail. Likewise, we shall compare our data with data that we have obtained from humans, for example, the responses to swine flu vaccination in a trial of 178 healthy adults which we co-ordinated.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The animal usage will be maximised by careful control of the breeding programme and fast turnover of genotyping information. The breeding programme will be set up so that we can obtain mutant and control mice at the same time, thereby maximising efficiency and the validity of the analysis. We will investigate the availability of genetically altered strains from the community, prior to generating any de novo. For new lines that need to be generated, we are employing novel techniques by which targeted animals can be generated within fewer generations (e.g.CRISPR/Cas9), thereby reducing mouse numbers. In many cases, the numbers of animals required will be reduced by longitudinal measurement of responses, by serial blood analysis or by optimised protocols for intravital imaging (see Refinement). Hence, the immune response to a challenge may be measured weekly in a set of six mice over a period of six weeks, rather than requiring six mice to be sacrificed weekly across that period. Among other things, such longitudinal usage provides essential information on

	the development or not of immunological memory.
3. 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.	In order to draw conclusions, and parallels from studying the interaction of immune cells with tissues, it is mandatory to use model organisms, which have a comparable complex tissue architecture and function. Mice with their rather short generation time, and their relative ease of husbandy, are considered such organisms. We strive to optimise animal welfare by working closely with BRF staff and monitoring animal wellbeing. Very many laboratories around the world have studied the immune system of mice over the past decades. This allows for a refined design of experiments based on published data, and at the same time the embedding and validation of our obtained data into the pool of knowledge, thereby reducing and refining the overall need for and approach to animal experiments. When we need to generate new lines, we will employ cutting-edge technologies to restrict a genetic modification to a specific tissue or cell type, for example by generating so-called conditional knockout mice. This will reduce the potential impact of genetic alteration on the whole animal, and will make our conclusions more robust. Unnecessary variation in the animal cohort will be minimised by use of gender and age-matched controls housed under identical conditions, as we have previously published, and likewise transgenic, knockout, and “knock-in” mice will routinely be generated or obtained on the same genetic background.

Project 15	**Immunological memory in transgenic mice**	
Key Words (max. 5 words)	T lymphocyte, virus, tumour, immunity	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Immunological memory is a characteristic feature of the immune system of mammals and is the goal of vaccination against infectious pathogens and cancer. Memory T lymphocytes are a type of blood cell that form the basis of immunological memory through their ability to rapidly eliminate invaders. How memory T lymphocytes develop is not well understood. The goal of the project is to study how newly discovered genes control the development of memory T lymphocytes, which give immunity to viral infection and cancer.	
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)?	The work in this project will lead to significant insights into a problem of central importance in medicine — what determines immunity to chronic viral infection? A stumbling block to the generation of vaccines based on T lymphocytes is the inability of immunization regimes to safely generate long-lived central memory T lymphocytes. Work for this project will identify new ways to overcome blocks that prevent successful immunization against viruses and cancer.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mus musculus 6900 over 5 years
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?	Expected adverse effects include unexpected susceptibility to infection or tumour challenge, increased morbidity due to incomplete bone marrow reconstitution after irradiation. Animals will be regularly monitored following treatment, more frequently if there are any unanticipated effects. Pain relief and/or other treatment will be administered as advised by the NVS. Where animals do not respond to treatment within a short time frame they will be killed using a humane method in order to reduce suffering. All procedures are either mild or moderate severity. All mice will be humanely killed by a humane method specified in this licence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Blood development requires multiple intact organs and so whole animal studies must be performed. Memory T lymphocytes cannot be generated in vitro and so requires an animal model. Invertebrates do not have lymphocytes and so cannot be used to study lymphoid development.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will employ pilot experiments with the minimum number of mice at each stage. In full-scale experiments, single doses of adoptively transferred cells and LCMV will be used to minimize mice numbers. Multiple data points will be obtained from mice by blood harvesting to maximize data per mouse. To minimize mice numbers, multiple immunological measurements will be performed on a given mouse. Unique genetically engineered mice will be cryo-preserved to eliminate the need for long-term breeding colonies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	Mice are the rodent of choice for the study of the development of the immunological system because of the availability of customised regents. In addition mice are preferred to other vertebrate species because of

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	their relatively rapid reproductive cycle. We will make use of our >20 years of experience in previous research which resulted in very refined models which provided accurate data with the minimum severity to animals. Extensive training to all researches undertaking the work will ensure a high degree of refinement. Mice will be killed using a humane method at the earliest possible time point in experiments to ensure the most reliable data with the minimum of suffering. Cryo-preservation of genetically engineered mice will avoid breeding.

Project 16	**Immune regulation, metabolism and tissue integrity**	
Key Words (max. 5 words)	T cells; environment, autoimmunity, mucosal immunity, metabolism	
Expected duration of the project (yrs)	2 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Investigation of the immune system has an impressive track-record bringing health benefits on a global scale, from eradicating and protecting against infectious diseases to promising new cancer treatments. However, the complexity of the system and many multicellular interactions mean that many questions remain, while new challenges continually emerge. How immune cells interact with and assist cells of barrier organs, the skin and especially the gut, and how these interactions contribute to overall health is the focus of this application. Our objectives are to make progress in answering the following questions: --How are immune cells, present at epithelial barriers, maintained? Specific subsets of immune cells are present only, or are highly enriched at, epithelial barriers. It is not	

	presently known how these cells are maintained or how they develop at barrier sites. -- How do conditions at epithelial barriers influence immune cell function? It is likely that specific conditions at barrier sites influence the composition and function of immune cells. One specific subset of white blood cells are called Th17 cells. These cells are highly enriched at epithelial barriers. We hypothesise it is the local environment which encourages the development of Th17 cells. The regulation of these cells is of importance since they are also involved in the initiation of auto-immunity. --How do immune cells and epithelial cells influence each other? Some immune cells, known as tissue resident cells, have very close interactions with epithelial cells. The nature of these interactions is not understood. It is however clear that these interactions have an influence on the epithelial cells and their function. We will investigate these interactions during the normal, non-triggered immune state as well as during inflammation using *in vitro* and *in vivo* infection models.
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 studies aim to reveal insights into the complexity of immune responses at epithelial sites. This will contribute to knowledge of how barrier health is maintained and of potential causes of disease, such as inflammatory bowel disease and auto-immunity. The interaction between immune cells and epithelial cells will contribute to a better understanding of immune-tissue cell interactions and could contribute to immune therapies, such as during inflammation as well as in the case of tumour immunology.
What species and approximate numbers of animals do you expect to use over what period of time?	The species of choice is the mouse. We expect to use up to 18000 mice over 2 years.
In the context of what you	The vast majority of animals we use will only show

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?	mild signs or less (up to 12550 animals). These animals are kept for breeding and maintenance of genetically modified mice and will be used to collect cell populations. Some populations are very rare and several donor mice will be required to obtain sufficient numbers of cells for our studies. Fewer than 5100 mice are expected to be subject to experiments that may lead to moderate effects, such as weight loss and colitis (the inflammation of the lining of the colon). These mice will be closely monitored and will be humanely killed if symptoms approach the maximum limit permitted for moderate symptoms (e.g., more than 20% weight loss). A maximum of 400 mice will be subjected to a severe protocol. This is a mouse model for multiple sclerosis in which mice develop progressive paralysis. The need for this is to assess the function of Th17 cells, known to be involved in this model. We have very successfully used this model for over 20 years to make important discoveries. There are several refinement measures in place to minimise suffering, such as special bedding to avoid sores when mice are immobile, aqua gel and mash to facilitate hydration and feeding. Due to the paralysis the mice do not appear to feel pain. Signs of paralysis start from around day 10 and during average experiments it will not be required to keep mice past days 30-35. Mice are closely monitored and checked daily. Mice on high paralysis score are checked for other signs of ill health. Mice suffering unduly will be culled within 24 hours.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Epithelial barriers as well as the many cells of the immune system are complex. Epithelial cells, which consist of different cell types, are in close contact with immune cells, which consist of many different cell types. In addition, microorganisms living at the outside of the epithelial barriers play an important role in maturation, development and function of the epithelial barriers and the immune system. Furthermore, the local environment with respect to

	metabolites and growth factors present are complex and not sufficiently understood. Some aspects of this can be re-capitulated to some extent *in vitro* using cell culture methods, which we will use wherever this is informative. However, we need to study the tissue in the animal to fully understand how it functions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We employ mathematical (statistical) analysis, e.g. of small scale pilot experiments, to ensure we use the minimum number of animals in each experiment to make solid conclusion while keeping suffering and animal usage at a minimum. For this, we are assisted by a dedicated biostatistician. In addition, we have many years of experience with the models proposed and make use of highly trained scientists.
3. 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 use the mouse, which is a standard animal of choice for medical research, especially research exploring cell and gene function. The mouse has been used for many years and good tools are available in this system (e.g. genetically modified animals, animals with reporter genes/cells and an array of good antibodies and recombinant proteins). The mouse genome is fully sequenced and we can build on a huge body of literature and knowledge. Genome sequencing has revealed that the majority of human genes have homologs in mouse with less than 1% of human genes lacking homologs in the mouse. Mice are relatively fast to breed and suitable to be maintained under laboratory conditions. We will minimise welfare costs; e.g. by housing mice infected with some micro-organisms on sand rather than grid flooring to minimise discomfort. We regular monitor mice, often daily, provide special bedding and floor-level access to food and water for mice experiencing any paralysis. In terms of general welfare monitoring, the Animal Usage Guidelines set out identifiers of discomfort and the Named Veterinary Surgeon is referred to any in cases of uncertainty.

Project 17	ZBTB proteins in lymphocyte development
Key Words	lymphoma
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;

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

Bcl6 is a gene that when mutated causes Non-Hodgkin's Lymphomas (NHL) such as diffuse large B-cell lymphomas (DLBCL) and follicular lymphomas (FL). In healthy individuals Bcl6 is essential for producing certain cells of the immune system that are required to fight infections, but mutations that cause the Bcl6 gene to go into 'overdrive' can transform normal cells of the immune system into lymphoma cells. i.e. Having 'too much' Bcl6 activity or having it at the wrong time can cause Non-Hodgkin's Lymphomas. The molecular mechanisms that control Bcl6 activity is still poorly understood, and understanding it is key to our ability to treat these types of lymphomas.

We have identified a novel protein that associates with Bcl6 and which could provide new insights into how Bcl6 works and how its activity can be targeted in lymphoma cells. The aim of this project is to understand the precise molecular mechanisms in which the novel protein we have identified associates with Bcl6 in the context of normal immune cells and in lymphoma.

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)?

This project could uncover new potential targets for pharmacological intervention in Non-Hodgkin's Lymphomas (NHL). Currently, treatments are scarce, and not all patients with lymphoma can benefit from them. It is also expected that this work will

reveal new insights into the developmental process of a normal, healthy immune system. This will have implications for our ability to regenerate the immune system in patients with congenital or acquired immunodeficiencies.

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

It is anticipated that this project will require approximately 6000 mice.

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?

The large majority of animals will be used for breeding and are not expected to suffer any discomfort.

Application of the 3Rs

Replacement

The normal developmental processes we are aiming to investigate cannot be faithfully reproduced in immortalised cell lines but they require the complexity of the entire immune system. Generating suitable genetic mouse models is therefore essential. As we build a clearer picture of the molecular mechanisms in which Bcl6 and associated factors are involved, we will attempt to model specific aspects in suitable cell lines if these exist, reducing the need for primary cells and therefore animals.

Reduction

In order to make sure the appropriate number of animals are used, statistical power calculations are generated based on guidelines for the design of experiments using laboratory animals (Festing MFW and Altman DG, 2002 ILAR J; Festing MFW, Overend P, Gaines Das R, Cortina Borja M and Berdoy M, The Design of Animal Experiments (2002) Laboratory Animals Ltd., London). We always seek additional expert advice from statisticians and mouse geneticists in order to make sure we use the minimal number of animals to answer our scientific questions within a 95% confidence interval.

Where possible we will isolate primary cells of several types from an animal with the desired genotype instead of breeding individual animals for separate tissues. This will maximize the efficiency of animal use.

Refinement

Mice represent a reasonable in vivo model system for studying the molecular mechanisms that regulate lymphocyte development due to the similarities with the processes taking place in humans. Previous work has also shown that mice are a suitable model to study the mechanisms of Bcl6-induced lymphoma. The data obtained from our approaches in animals will therefore be able to inform studies we intend to carry out in human lymphoma cells extracted from patients.

Suffering will be minimised by using mice mainly as a source of primary cells for in vitro experimentation, and this project requires no invasive procedures be performed on the animals in their lifetime.

Genetic manipulation experiments will be refined by the use of conditional alleles, which means induced mutations will only affect specific cells of the immune system while the rest of the animal tissues will be protected. This means that the majority of animals used in this project will not suffer any adverse effects in their lifetime as they will be kept in a protective environment that prevents infections even in the absence of a fully functional immune system.

Project 18	Immune cell regulation of epithelial damage and repair
Key Words	Epithelial, Repair, T cells, auto-inflammation
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;

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

Our objective is to learn more about how cells of the immune system regulate the epithelial cells of the gut and skin. Epithelial cells make up the external barrier of your body, they are in constant interaction with immune cells, such as T cells, that live in large numbers in the gut tissue and skin. The role of epithelial cells is to protect the body's internal environment and control what can and cannot enter. The epithelial cells in the gut and skin are being constantly replenished, therefore proliferation of epithelial cells must be tightly controlled to ensure a supply of new cells to replace old cells and to respond to any tissue damage caused by inflammation or mechanical damage. Auto-inflammatory diseases such as irritable bowel disease in the gut and dermatitis in the skin result in and are made worse by damage of the epithelial barrier layer. The immune cells present in the gut and skin become activated and fight the microbes that have invaded the body through the damaged epithelium, but this is not their only role, they also help regulate epithelial repair processes. The incidence of these auto-inflammatory diseases is currently on the rise, therefore identifying pathways that immune cells use to regulate epithelial behaviour and drive repair is a step towards identifying targets for treatment of disease. Our work aims to identify and learn more about the pathways immune cells use to drive the repair of epithelial tissue which has been damaged during auto-inflammatory diseases such as IBD and psoriasis.

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)?

The benefits of this research will be to advance understanding of the mechanisms immune cells use to regulate epithelium both preceding and during damage and inflammation and the significance of these interactions in orchestrating epithelial

repair. Ultimately understanding how these pathways work could contribute to the design of future strategies to therapeutically limit epithelial damage and drive repair in diseases where epithelium is damaged.

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

We will use mice that may be genetically modified in order to understand the role of specific cells and pathways in immune regulation of epithelium. A maximum of 7350 will be used over the 5 year lifetime of this project. Most of the animals used are for the purpose of generating the genetically altered mice needed.

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?

Part of the project requires the breeding of genetically modified mice with specific alterations to the immune cells or epithelium of the gut or skin. We breed the mice in highly protected environments to avoid unwanted health problems. For all our studies appropriate control measures are in place to ensure that all animals will be monitored closely, and appropriate action taken. Animals will be carefully monitored for deviations from normal behaviour that might indicate pain or suffering and our named veterinary surgeon consulted if this were the case. All animals will be killed at the end, either by terminal anaesthesia or an approved humane method. Our studies of auto-inflammatory bowel disease uses the DSS colitis model, adverse effects are loss of body weight, diarrhoea, intestinal bleeding, these are followed by a period of recovery and repair. We consider that these colitis studies will never extend beyond moderate severity. Adverse effects of our Psoriasis and atopic dermatitis models are skin thickening, flaking, scabbing and redness, followed by a period of recovery and repair. We consider that these psoriasis and dermatitis models will never extend beyond moderate severity. Our standard murine adult skin lesions are generally re-epithelialised in approximately 7 days and appear to cause little discomfort. We consider these wounding studies will never extend beyond Moderate severity.

Application of the 3Rs

Replacement

Both gut and skin are complex structures and responses to damage and auto-inflammatory diseases involve a complex interplay between the epithelial cells, cells of the immune system and the microbes which are resident in these tissues. Despite efforts to carry out replacement strategies using organ cultures, not all of the cell types involved in vivo can be successfully grown in vitro and therefore the damage resulting from auto-inflammatory diseases and immune responses cannot yet be fully replicated in vitro. Consequently animal models are still required to study the mechanisms of human colitis, skin inflammation, damage and repair.

Reduction

For all of our studies we use the minimum number of animals possible to provide rigorous, statistically significant data points, based on power analysis and previous work. We consult with colleagues doing similar experiments and statisticians about appropriate numbers for our studies. Results will be monitored as experiments are undertaken to determine whether subsequent experiments could use fewer animals if possible.

Refinement

Mice have been chosen for these studies because their immune cell biology currently provides the best and most highly characterized model for understanding human immunity. In addition genetic manipulation of the genes of interest are available in mice, and models for both human colitis and skin inflammation are well established in the mouse. The use of both conventional inbred and genetically inbred mouse strains minimises variability in the responses between individuals; thus ensuring that fewer animals are required as a result.

A key objective of our studies is to study epithelial repair. Therefore all the models selected are mild enough to enable recovery and repair following withdrawal of the disease causative reagent.

We always question whether or not the potential benefits justify any suffering and whether answers could be achieved using in vitro culture systems. Whenever experiments are performed which involve any degree of distress or potential pain, we routinely check the procedures in order to refine them further for future studies so that these effects may be minimised. This will continue to be our policy in the future.

Project 19	Molecular mechanisms of T cell mediated immune responses
Key Words	Immune response, autoimmunity, Egr, viral infection, tumour
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;

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

The overall aim of the project is to understand how two molecules (Egr2 and 3) affect the functionality of T lymphocytes, and whether this can be optimized to treat virus infections and cancers, with the potential of developing a new therapeutic vaccine.

The immune system is responsible for protecting our body from invading pathogens. However, in autoimmune diseases, the immune system mistakes one's own tissues for pathogens, and hence the body begins to attack itself. In contrast, after contracting chronic infections such as hepatitis C, the immune system becomes too weak to attack the invading virus. It is unknown why these diseases cause the immune system to over-perform or under-perform.

However, we have recently discovered that the excess production of two proteins, Egr2 and 3, in T lymphocytes (a type of white blood cell), results in weaker anti-viral responses. Conversely, limiting the production of these proteins leads to the development of autoimmune diseases.

To correct the malfunction of T lymphocytes in these diseases, we must understand the mechanisms of Egr2 and 3.

By using unique mouse models, the objectives of this project are;

1. To discover the mechanisms of Egr2 and 3 in T cells at a molecular level.

2. To understand the function of T cells under conditions of virus infection, tumours and autoimmune diseases, in mice that are either genetically missing Egr2 or 3 or over producing Egr2 or 3.

3. To evaluate the effectiveness of regulating Egr2 and 3 production in T cells for treating autoimmune conditions.

4. Finally, to reconstitute immune activators into artificial cells (Nano-APC), and use these to correct the function of T cells in autoimmune diseases and chronic infection.

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)?

The outcome of these experiments will make an important contribution to the development of new methods for treating autoimmune diseases, as well as new vaccines for cancer, which are currently incurable.

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

Mice. Over the duration of the project, it is envisaged that approximately 4000 mice will be used, including those that are used only as breeding animals.

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?

All of the proposed experiments are well established and used by many laboratories for more than 20 years. There are expected adverse effects from Experimental Autoimmune Encephalomyelitis (EAE), vaccinia virus infection and implanted tumour and irradiation to destroy bone marrow for bone marrow transplantation experiments. In EAE conditions, mice will have weak movement of tail and limbs.. Weight loss is the major sign of infection. There will be no signs of adverse effects in tumour bearing animals as long as tumours are less than 10mm3.. Irradiation has limited and short period of adverse effects such as less movement that may last for a few hours after irradiation. Once these signs are observed, mice will be humanely killed at the earliest sign possible.

Application of the 3Rs

Replacement

Immune function is established in living organisms. In addition, the method for investigating the function of any given molecule is to genetically modify the molecule in animals. We cannot carry out life experiments in humans.

Reduction

- We will assess some molecular mechanisms using cells after adding or removing Egr2 and 3 genes in the laboratory.

- To minimise the use of animals without affecting scientific results, we will design the each test with accurate number of animals which will be just enough to give us clear results. We will carry out, if possible, pre-experiments on cell lines to optimize the procedures in order to achieve 100% success of the animal experiments.

Refinement

- We have chosen to use mice since the parallels between the mouse and the human immune systems are well understood and mouse models of the diseases well established. This means that reagents are readily available and alleviates the need to establish novel models which greatly reduces animal use.

- The newly established GFP-Egr2 knock-in model has proven to be generally normal without any health problems. It give us a great advantage for fulfilling the objectives under this new PPL, while greatly reducing the need of Egr2 KO model which showed some welfare issues with breeding difficulties, thus also minimising welfare costs for the animals.

- We apply well defined experimental techniques with minimal intervention to avoid distressing the animals, expert preparation of samples for investigation, strict adherence to protocols and keep the time that an animal is under experimentation as short as possible

Project 20	Lymphocyte development and antibody repertoire formation
Key Words	Fighting infection, Antibodies, Ageing, Immune response, White blood cells
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;

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

The aim of this project is to understand how white blood cells termed B cells produce antibodies to fight infections. This process is impaired in older people for unclear reasons. The objectives are to determine which processes are perturbed in the nucleus of B cells (the part of the cell that contains the DNA), in order to identify ways in which the immune system could be boosted to fight infection more effectively. These processes include rearrangement of DNA to make millions of antibody types, and activation of genes that allow B cells to grow and multiply. We will also study earlier events that lead to production of fewer B cells in older people, and defects in accessory cells that support B cell growth to provide wider insights into defects in B cell function.

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)?

Key factors and mechanisms that are essential for encoding millions of antibodies will be identified and functionally validated. This will increase our understanding of how the body fights infection, and may provide candidates to test as immune system boosters (eg for incorporation into vaccines to make them more effective for older people).

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

We expect to use approximately 7000 mice over a 5 year period. These will include genetically altered mice and control mice. Approximately 1000 of these mice will be wild-type mice aged to 21-24 months to study alterations in B cell function in ageing.

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?

We plan to remove tissues containing B cells and accessory cells from mice that have been humanely killed. Thus we do not expect any adverse effects and the expected level of severity will be mild.

Application of the 3Rs

Replacement

We need to use animals because B cells develop in the bone marrow and rely on several accessory cells and factors in a dynamic manner that is not well understood and cannot be fully replicated in ex vivo culture systems. Furthermore, to understand the effects of ageing on B cell function, it is essential to allow the B cells to be exposed to all relevant influences over an extended lifespan. As these include other influences from the whole body, which are poorly understood, we cannot yet incorporate all of these into an in vitro system.

Reduction

We will consult our Biostatistician to ensure we use the minimum number of animals that will provide statistically valid data. We are using genome-wide next generation sequencing approaches which provide data on all the genes in the genome simultaneously as an alternative to studying subsets of genes. In this way we require fewer experiments, which are now being replaced by extensive bioinformatics analysis.

Refinement

We use mice because these animals have an immune system, including B cells, that closely resembles the human system. In particular, the DNA sequences used to make antibodies and the mechanisms that underpin this process are highly conserved in evolution. Therefore, mice have been widely used to study the immune system and there are several well-established genetically altered mouse models that we will be able to avail of, as well as well characterised reagents eg for purifying B cells. Additionally, mice are a well-established model for studying human ageing, since they exhibit most of the major ageing alterations experienced by humans. To minimise welfare costs, mice will be housed in our state of the art animal facility under sterile conditions that prevent infection, under the care of experienced and highly qualified animal staff, and with strict adherence toHome Office guidelines, overseen by our Animal Welfare and Ethical Review Board (AWERB).

Project 21	DNA double-strand break repair, immunity & cancer
Key Words	DNA repair, Cancer, Immune system
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:

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

DNA repair defects in humans can in some cases lead to immune system failure and in other cases an increased susceptibility to develop cancer. This project builds on some of our preliminary findings that the same DNA repair mechanism that is used to generate genetic diversity in the cells of our immune system (lymphocytes) is additionally responsible for generating the chromosomal damage and mutations that triggers tumourigenesis in common hereditary breast and ovarian cancer. A primary aim of this project is to develop a mechanistic understanding of the DNA repair machinery that is typically used by mammalian immune systems to generate different classes of antibody, and then questioning whether the proteins that make up this machinery also play a role in cancer development, or cellular responses to important classes of anti-cancer drug. To date, the cellular mechanisms and DNA repair proteins involved in immune responses and cancer development are poorly understood, and our research aims to fill this void and help Scientists understand the links between normal immune function and cancer.

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)?

This work will benefit Patients and the health service. Breast cancer is by far the most common cancer among women in the UK (2010), accounting for 31% of all new cases of cancer in females. It affects over 50,000 people/year in the UK and is responsible for more than 11,500 deaths/year. Our original route into this research came from an anti-cancer drug resistance perspective relevant for understanding patient responses to anti-breast cancer therapies. In tumour models, Rev7/53BP1-loss renders breast cancer cells resistant to what normally represent highly effective therapies. These findings may therefore highlight mutations that are selected for during cancer evolution and therapy regimes. Although one would predict that the

identification of such mutations in human cancer would lead to poor patient prognosis, they would at least help better predict responses to secondary treatment regimes, and help avoid the administration of futile treatments that result in patient suffering without giving therapeutic gain. Commercial beneficiaries such as the pharmaceutical industry may also benefit. We also hope that our aim to understand the basic molecular mechanisms underlying tumourigenesis and drug resistance may facilitate the identification of compensatory pathways in cancer. These might then be targeted to selectively sensitise sub-classes of cancer in modern personalised medicine approaches.

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

We will use the laboratory mouse, and anticipate we will use no more than 12,000 animals over the 5 year licence period.

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?

We will breed genetically modified transgenic mice with the aim of obtaining primary cells from harvested tissue of schedule 1 killed mice. These cells will then be cultured ex vivo so that we can look at how DNA repair occurs in normal immune cells upon their activation by cytokines and antigens. Most procedures will be mild in severity as we will not be performing in vivo experiments, however we have incorporated a breeding procedure of moderate severity in contingency that the genetic mutations we generate in mice lead to unforeseen phenotypes or cancer pre-disposition. For immune experiments, once mice of the required genotypes have been obtained in breeding experiments they will be killed using a schedule 1 method before tissues are harvested for cell isolation. Otherwise animals exhibiting any unexpected harmful phenotypes will be killed, or if scientifically valuable advice will be sought from the Home Office inspector and mice will be sacrificed at predefined and humane endpoints.

Application of the 3Rs

Replacement

The immune response involves multiple, complex systems interacting in a physiological environment, which cannot be replicated in tissue culture, and so there is no adequate alternative but to employ animals in these studies. Cell culture systems will replace animal tissue in our experiments to characterise the biochemical effects of mutations; but transformed cell lines do not accurately recapitulate the properties of primary immune cells *in vivo* or those harvested from tissues and cultured *ex vivo.*

In this project, the majority of experiments will be performed using primary cell isolates following purification from the primary tissues of schedule 1 killed mice. Such experiments will therefore replace the use of live animals in experiments.

Reduction

We have used statistics to ensure that we use the minimum number of animals to obtain scientifically meaningful data.

In breeding experiments, genetic crosses will be carefully designed to obtain the maximum number of useful animals with the minimize wastage.

When certain transgenic mice strains are no longer required for experiments, they will be cryopreserved to reduce the numbers of mice used in unnecessary breedings.

Refinement

The laboratory mouse is the species of choice for studying immunology and deriving primary tissues, and the ideal mammal for genetic studies where animals need to be generated rapidly. Using mice provides us with an opportunity to study the role of genes during immune responses and examine its role in genome regulation in well-defined primary tissues, which is not possible in higher organisms. The similarity of human and murine immune systems is reflected in homology at a genetic and protein level, making mice a good model for understanding the equivalent biological processes in humans. For example, one protein we seek to study, Rev7 is highly conserved between man and mouse, sharing 98.5% sequence identity.

Attention will be paid to animal husbandry, including the provision of environmental enrichment and co-housing animals. Animals exhibiting any unexpected harmful phenotypes will be killed, All adverse effects will be documented and periodically assessed in order to detect sporadic unexpected events. The risk of adverse phenotypes in transgenic mouse strains will also be reduced through use of conditional-knockout alleles in strains harbouring secondary alleles to direct tissue specific *Cre*-expression. By enabling tissue-specific gene inactivation, such as our proposed use of the Mb1-cre strain to conditionally inactivate DNA repair genes such as *Rev7* in early B-cell precursor cells, we will minimise the potential of harmful phenotypes that might occur as a result of gene inactivation in other tissues.

Project 22	Interactions between mast cells and helminths in inflammatory disease
Key Words	mast cells, helminths, diabetes, arthritis, cardiovascular disease
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	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

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

The purpose of this licence is to identify the role that mast cells play in the induction, perpetuation and amplification of immune responses in inflammatory, infectious and allergic disease and thus lead to the development of new medicines. We will carry out research into the fundamental mechanisms by which mast cells trigger or control inflammation so as to reveal novel targets for therapeutic intervention. We will then identify biological and chemical reagents with the potential to control and prevent inflammatory diseases. We also aim to evaluated novel compounds for anthelmintic activity.

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)?

The project will lead to greater understanding of the immune processed that lead to a range of inflammatory diseases. It will help to identify novel therapeutic reagents for more effective treatment against some of the most important inflammatory and infectious diseases that impact on the health and economy.

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

Mice, 3,400 (680 per year)

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?

The most likely adverse effects for all procedures are moderate as assessed by weight loss of less than 10% in comparison to age matched controls. Where appropriate analgesia will be used. All animals will be euthanised at the end of the experiment. Any animals displaying deviation from normal health, other than due to the inevitable effects of the procedure, will be promptly euthanised or referred for veterinary attention, the former being the more likely course of action. Parasite infection may result in mild gastrointestinal discomfort for a maximum of three weeks (weight loss, hunched posture and piloerection). Moderate muscle discomfort may result (altered gait, reduced movement and abnormal posture) however these effects should be rare. Very rarely, animals may also show respiratory distress (shallow, fast breathing) from migration of larval stages of the parasite this effect should be transient (< 48hours). The dose of parasites to be used will be adjusted so as to limit the effects of the infection, although the gastrointestinal and muscular effects are an inevitable consequence of the infection. Animals developing arthritis may show signs of ill health, e.g. listlessness, hunching, weight loss. Food and water will be placed with easy reach. Extra bedding and nesting material will be added for comfort. At the peak of the inflammatory response animals may have severe loss of limb movement for a maximum of 7 days. In a typical experiment, less than 10% of the animals are euthanised because of adverse effects. The development of diabetes is characterised by polyuria, polyphagia, and polydypsia, disease will be confirmed by measurement of blood and urine glucose levels. Mice in the later stages of overt diabetes may exhibit weight loss, hunching and immobility. Once diabetes is confirmed mice will be sacrificed by a Schedule 1 method. For the study of cardiovascular disease ApoE knockout used have a high circulating cholesterol level, however, this rarely causes adverse effects. Dietary manipulation will not cause weight loss but could cause weight gain which should not affect the health of the animal. If any health problems arise the appropriate treatment will be given.

Application of the 3Rs

Replacement

Our study will begin with the investigation of the fundamental role of mast cells in the development of inflammation. Extensive *in vitro* studies will be conducted initially using established cell lines or isolated murine white blood cells to identify the immunological pathways that may lead to inflammation. It is essential to conduct these studies in animal models because although preliminary experiments can be done in vitro, this does not reflect the complex interactions which take place in a whole animal during disease development. All the animals used in this project will

be mice as they are the lowest species which have an immune system similar to that of humans. Furthermore, a range of reagents and genetically modified strains of mice are available to provide more definitive answers to the questions addressed

Reduction

Statistical advice has been sought and will be used to ensure that the minimum number of mice used will be consistent with the aims of the protocols while achieving statistically valid results. Techniques such as in vivo imaging permit multiple analysis on single animals thus reducing the numbers required.

Refinement

Animals will be group housed in cages which permit free movement and contain environmental enrichment appropriate to their species. Husbandry and care procedures are based on best practice, and regular monitoring will be conducted by highly trained staff. In all cases, the endpoints of the experiments will be measurements acquired from tests which are considered minimally traumatic to the animals and are of short duration. Pilot studies are planned wherever necessary.

Project 23	Complement properdin in immunity and inflammation
Key Words	Properdin, tumour, diet, stimulation
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:

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

This five-year-work plan sets out to on the one hand deepen understanding in inflammatory processes that occur as part of localised reactions or complex diseases such as autoimmunity, allergy, tumour immunity, on the other hand to determine how therapeutic approaches are influenced by factors we can easily model in mice. Both aims are intended to inform considerations on how to improve treatment of patients. Based on expertise within an area of immunity, research is centred on complement properdin and uses knowledge, established methods and new collaborations to deliver on the program.

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)?

The work will increase understanding of complement properdin in inflammation. Inflammation is s a key pathogenic process. This project has particular interest in tumour immunology, respiratory allergy and fatty liver disease.

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

In house bred genetically altered mice with their wildtype controls will be used over a period of five years. A novel line will be generated by crossing of existing genetically altered mice with the intention to improve autoimmune disease. Up to 4500 animals will be used over the five-year duration of the project.

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?

Most of the genetically altered mice used in this project have no harmful phenotype, but one mutant line has autoimmune disease (e.g. MRL-Faslpr), affecting skin and kidney function. The wellbeing of these or similar mice, state of their skin (rash) and biochemical measurements of renal impairment will be tightly recorded, and decision for killing at humane endpoint communicated swiftly. The types of high fat diets chosen do not impact on welfare in the timeframe of the protocols. Tumour sizes will be recorded; they do not impact on movement and behaviour. Mice are typically killed as a group when the stipulated size limit is reached. Allergy and hock inflammation are short protocols, and adverse effects not expected. At any sign, therefore, mice will be humanely killed. The cumulative severity will vary between mice and will be diligently recorded, as some mice will receive “optional” procedures. All animals will be killed after their procedural sequences and none will be re-used.

Application of the 3Rs

Replacement

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

Replacement

Wherever it is scientifically reasonable to use human material, commercially available cell lines or tissues and primary cells from non-experimental mice for *ex vivo* analyses, this is the first choice. Where the work addresses involvement of the immune system, however, the whole organism is needed to obtain an experimental model with which principles can be studied that will aid in the understanding of health and disease.

Reduction

NC3R’s Experimental Design Assistant will guide the flow of the experiment, identification of relevant variables to consider and pilot experiments to undertake. To reduce variation, matched (age, sex, diet) mice, preferably within their litters, are used for comparisons. Imaging of mice may serve to reduce experimental numbers.

Refinement

Central to the work programme is to further characterise the role of properdin in disease processes relevant to man. This can be best achieved by the use of a properdin-deficient mouse line, which proved instrumental in proving that the complement controlled cellular activity primarily drives the response to inflammation and its outcome, not the blood borne capacity to activate complement. Our improved analytical methods can capture early responses in our tumour model; our hock edema model avoids more severe models using paw or ear; duration of high fat diet is currently reviewed to be reduced to five weeks; we participate in measures that rejoin male exbreeders, avoiding single housing.

Project 24	Inflammation, cell death and cancer
Key Words	Inflammation, immunity, cancer
Expected duration of the project	0 year(s) 3 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:

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

Cell death and inflammation is tightly controlled in the cells from our body in physiological conditions. Upon infections or mechanical damage our immune system reacts to elicit an inflammatory and immune response against that damage and the tissue repair system will eventually restore normal conditions.

Cell death, whether it is by its occurrence or prevention, is an important event in this process. If these events are activated in sterile conditions or in absence of any exogenous stimulus our body can react against itself and cause disease. Deregulation of the signalling pathways can lead to autoimmunity, autoinflammation and cancer. Still, the mechanisms leading to this deregulation are poorly understood. In order to develop new therapeutic strategies for these diseases, it is imperative to characterize the key components of these pathways, their activities and how they interplay. To this end we will study how inflammatory and immune pathways crosstalk with cell death and genes controlling oncogenic transformation. In addition, we intend to evaluate the effect of potential inhibitors for specific proteins or activities within a protein that are key for the regulation of such pathways.

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)?

In order to prevent or reverse life threatening diseases such as chronic inflammation, autoimmunity and cancer new therapeutic treatments are needed. The use of genetic mouse models is crucial to understand these pathways and to test potential inhibitors that could increase human and animal welfare and even be life-saving.

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

In order to achieve this we will need approximately 1750 mice over a period of 3 months.

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?

Some of the animal models we intend to use spontaneously develop dermatitis. If we are not able to revert this condition by genetic experiments or chemical/biological treatments the animals will be humanely killed as soon as they start to show signs of distress (hunchback position, excessive scratching, etc). Other animals develop swollen lymph nodes; this condition is not harmful for the animal and they will be either bred or used in experiments. Animals will be humanely killed as soon as enlarged nodules restrict movement or any other physiological condition. Some other adverse effects may arise by the application of chemical or biological substances into the mice. The animals will be closely monitored during these procedures and will be humanely killed as soon as they start to show signs of distress (hunchback position, lack of grooming, excessive loss weight, etc).

Application of the 3Rs

Replacement

All of the planned in vivo work is generally preceded by intensive in vitro studies. Cell lines have been generated from the transgenic mouse strains mentioned above. Human cancer cell lines with defined genetic alterations are also present. They will be monitored for all the physiological conditions that are possible in vitro. Although it can provide important molecular and cell physiological insight and a justification for further in vivo work, in vitro work cannot fully recapitulate the pathophysiological situation in the tumour microenvironment. In addition, the evaluation of the morbidity caused by inflammation in vivo is irreplaceable with the in-vitro studies since the cell lines do not completely recapitulate the inflammatory circumstances in the tissues.

Reduction

Preceding in vitro experiments (see above) are going to limit the number of animals required for the in vivo investigation as key components involved in inflammation and oncogene-driven cancer can be identified in cell lines.

Mouse colonies will be closely monitored by members of the group to avoid excessive breeding.

For every in vivo experiment we write a protocol which includes a statement of the objectives, a description of the experiment listing experimental treatments, number of mice per treatment group and the experimental material needed to make sure we

can reach statistical significance without the need to repeat the experimental protocol with more mice.

Refinement

Experiments will be mostly conducted using inbred and gene-targeted mice on a C57BL/6 background, which is the most common and well described strain of mice. The standard protocols have been optimised for this species which also reduces the number of mice needed for optimisation. In general, we intend to minimise suffering of the GA mice by breeding conditional knock-in and knock-out mice. This means, mice are generally only going to develop a phenotype if backcrossed to tissue-specific and/or inducible Cre-recombinase-expressing animals. Therefore, in the breeding steps preceding the last step required to generate the necessary experimental groups the mice will be phenotypically healthy. Cre-recombinase is only crossed in at the last possible breeding step. On the basis of our recent in vivo research, whenever possible we now maintain otherwise sick animals with a combination of mutations that either delays or prevents the onset of disease. Regarding the cancer models, a significant tumour burden is only going to develop in the final experimental groups. Animals no longer needed for breeding or where any of the symponts mentioned arises will be culled by the appropriate Schedule 1 method. Concerning the morbidity tests following the induction of acute inflammation, the use of preliminary experiments will be performed to determine the doses, morbidity, time course of effects, and frequency of observations and to set an earlier and appropriate endpoint.

Project 25	**Translation of the immunological synapse**	
Key Words (max. 5 words)	Immunological synapse, Tolerogenic DCs, Central tolerance, Atopic dermatitis, Original antigenic sin	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	×	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project investigates the way immune cells interact with each other. These are dynamic processes, which transfer information between different cell types and are vital for generating an immune response. We seek to understand and characterise these interactions in infection, allergic inflammation, autoimmunity and cancer, situations in which the immune system plays a large role. This involves characterising such interactions at the level of cells, tissues and whole organisms with methods that allow us to follow interactions as they occur.	
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)?	Cell biological studies have already led to better understanding of how antigen specific communication between different immune cells is carried out. Autoimmunity is a major problem in the developed nations including the United Kingdom	

	with prevalence of rheumatic arthritis at 1.25% and ankylosing spondalitis at 0.5%. It has been shown that both genetic and environmental factors are involved in the pathogenesis/ development of these diseases. A burning question is how autoreactive Tcells are generated in the first place and how do they overcome tolerance ultimately resulting in responses against self-tissue.
What species and approximate numbers of animals do you expect to use over what period of time?	This project license involves the use of mice (wild-type and genetically modified). Breeding of these mice is expected to be 12400 over five years.
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?	Potential adverse effects would include local inflammation, weight loss and pain or irritation from substances injected, subcutaneous tumour growth, or skin rash and diarrhoea from irradiation and reconstitution. Animals that demonstrate a deviation from normal health, as assessed by food and water intake, weight, behaviour and general appearance will be closely monitored and killed as a humane endpoint.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The nature of studying immune cell communication requires consideration of the local environment in which the interactions take place (microenvironment). This complex mixture of factors supplied by various cell types within a tissue shape immune responses, making it essential to evaluate such hypotheses in whole body/tissue systems. The complexity of the diseases of interest such as atopic dermatitis, which has a strong environmental component, cannot be faithfully replicated without a whole-animal approach. While systems involving self-propagating, modified cells in vitro exist, these behave in a

	substantially different, and less physiologically relevant, way to cells that could be sourced from genetically modified animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Most of the planned experiments require a group size of 5-6 animals. Many of our immunological assay read-outs can have a wide variation of results within a group of similar animals. Group size of 5-6 is commonly used in the reported literature for most of our experiments, based on power calculations. Every effort will be made to reduce the number of mice required. This will be achieved by use of early 'pilot' experiments to understand intra and inter-group variation and optimization of experimental design e.g. including age and sex matching mice used to reduce variation in data. Reduced variation will allow us to use fewer animals to test our hypotheses. Maximum use of tissue obtained from animals will be ensured to further reduce the required numbers, and tissue samples will be archived.
3. 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 choose mice as the appropriate model organism for the proposed experiments because this species provides greatest flexibility in terms of availability of genetic modification, reagents and previous data. This provides unique opportunities to study certain immune cell interactions in a rodent system which shares many of the same features as the human immune system. In infection work, sublethal doses of infectious agents will be supplied and define cut off points for all work will avoid unnecessary animal suffering. The use of genetically modified mice will reduce the requirement for application of labelling substances to visualise immune cells which will reduce frequency of injections. Inhalation anaesthetics will be used where possible to avoid risks associated with injectable

	forms (complexity in maintenance, dose control). In the case of skin-inflammatory models, the site of interest permits less physiological stress in recovery surgery i.e. no surgical intervention is performed, further aiding animal wellbeing and reducing required numbers. We have elected to image undisturbed mouse ear skin, this obviates requirement for surgery which reduces suffering, time under anaesthesia and confounding inflammation. Any mouse showing clinical signs of pain or distress will be killed as a humane endpoint to minimise suffering.

Project 26	**Immune activation in health and disease**	
Key Words (max. 5 words)	Immunity, adjuvants, vaccines, allergy, hypersensitivity	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Immunological adjuvants activate the immune system, and are used for improving human health as part of vaccines. However very few adjuvants are available for human and veterinary vaccine use, mainly because they are difficult to license for safety reasons. We do not sufficiently understand how adjuvants work to be able to improve them. **Objective 1** is therefore to understand the mechanism of action of new adjuvants to improve their efficacy and safety for vaccine use. Some adjuvants may be created under oxidative conditions, such as in industrial pollution or when food is heated to high temperatures, and may cause harmful immune activation leading to immunological disease such as allergy and asthma. **Objective 2** is therefore to understand how environmental immunological adjuvants may trigger unwanted immunological disease, and how we may reduce this. **Objective 3** is to test specific new vaccine approaches to protect from influenza virus infection,	

	and will use a model in which mice are vaccinated, then challenged with live influenza virus to see if they are protected from disease.
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)?	Understanding how adjuvants trigger immune responses at the cellular and molecular levels will help us to both develop better vaccines for clinical and veterinary use, and to avoid or reduce some immune system-mediated diseases including allergy, asthma and some hypersensitivities.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 5400 inbred and GA mice over 5 years
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?	**Objective 1**. Will involve administration of immune adjuvants and other substances via different routes that mimic human or animal vaccination routes. Any rare potential adverse effects are likely to be localised to the site of vaccination and might include short-term swelling, redness and irritation. Discomfort suffered by the animals is most likely to come from giving them multiple vaccinations in a small number (<20%) of experiments. It is not expected that any of the experiments will induce any disease or harmful side effects. This objective should mostly have only mild adverse effects and will not exceed moderate severity for any of the experiments, at the end of which the animals will be humanely killed. **Objective 2**. Mouse models of immune priming for allergy, asthma hypersensitivity and autoimmunity. These models are not designed to elicit the actual disease associated with these conditions, but only to generate the immune responses associated with these conditions. Therefore there are unlikely to be any adverse effects beyond multiple exposures to the immune activating substances, and potentially on rare occasions very mild forms of the diseases. This objective should mostly have only mild adverse effects and will not exceed moderate severity for any of the experiments, at the end of which the animals will be humanely killed.

	Objective 3. The mouse model for influenza vaccine efficacy based upon infecting mice with live influenza virus. The major adverse effect expected from these experiments is the disease caused by the virus infection, which results primarily in weight loss. We have reduced the level of weight loss that is acceptable to 15%, beyond which we will humanely kill the animal. This objective will not exceed moderate severity for any of the experiments, at the end of which the animals will be humanely killed. We will breed a small number of normal mice and mice with non-harmful genetic alterations to answer specific questions about mechanisms that lead to immune activation in our experiments. This will not exceed a mild level of adverse effects and mice will be humanely killed at the end of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to mimic the complex nature of the human immune system *in vitro*. For example, we cannot evaluate vaccine-mediated immune activation and efficacy without using living mammals. Immune system activation requires the complicated interaction of many cell types in living tissues that cannot be mimicked outside of living animals. Studies of immune disease such as allergy and asthma likewise require analysis in the context of an intact mammalian host. However we will carry out analyses wherever possible using cells and tissues to complement and where possible reduce animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use inbred mice as their identical genetics means that their immune system mostly behaves in the same way within a population. This reduces variation between how individual mice respond, allowing reductions in group size whilst maintaining statistic power. Where possible we will base our use of animal numbers on past experimental data from our laboratory and from other laboratories, to obtain maximum statistical power with the minimum number

	of animals. For each new experiment to be performed we will consider our existing database and if appropriate consult with an expert biostatistician to reduce animal numbers and groups with appropriate controls. We will review our in vivo models, and if another model allows smaller animal numbers without reducing quality of the results obtained then we will adopt that model.
3. 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.	Analysis of how the immune system is activated in vaccine design (objectives 1 & 3) and immune pathology (objective 2) cannot ethically be carried out in humans, and cannot meaningfully be carried out in non-mammalian species as their immune systems are too divergent from man. Inbred mice will be used as they are immunological highly characterised, generically identical, may have specific genetic alterations. However human immune cells will be used for complementary *in vitro* analyses. Immune activating agents with known deleterious effects will be replaced by other safer agents. Mouse strains that are genetically and phenotypically sensitive to allergic or hypersensitive priming will not be used for our models to avoid severe symptoms such as anaphylaxis. Models for allergy and other immune diseases will be modified over the course of this license to reduce any potential for animal suffering. During procedures in which animals are at risk of adverse effects, such as post-influenza virus challenge or post-allergy challenge, we will monitor the animals intensively until the period of risk is over, until the humane endpoint is reached, or until the end of the procedure and the mice are humanely kille

Project 27	**Mouse models of chronic inflammatory diseases**	
Key Words (max. 5 words)	Cancer, infections, autoimmune disease, pain	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Inflammation alerts the body's defences – called the immune system - to the presence of potential harmful threats. Threats include germs that cause disease and death unless the immune system seeks out and destroys them. However, inflammation also occurs in other clinical syndromes, including cancer, autoimmune diseases, after surgery to transplant life-saving organs and tissues, after exposure to radiation and sunlight and in response to wounds, burns and toxic chemicals or natural substances such as poison ivy, airborne allergens or some foods. Inflammation is a complex process involving many cells that interact and communicating using a variety of signals. Our research goals are to improve understanding of inflammation and to develop new treatments for the syndromes listed above. To achieve this, human clinical syndromes are modelled using mice. Mice are treated with defined reagents, cells, pathogens, radiation, or given transplants to induce inflammatory responses. Once induced, we study how inflammation impacts physiologic responses that cause disease or	

	transplant rejection. New knowledge is used to develop novel treatments to prevent disease or transplant rejection. Mice are useful for this work because many genetically altered mouse strains are available. This allows us to pinpoint genes and proteins that control inflammation and immune responses to inflammation. A very puzzling aspect of inflammation is that turns the immune system on or off in different circumstances. For example, inflammation caused by infections stimulates immune responses but inflammation associated with developing cancers inhibits immunity. Our work builds on an original discovery that developing foetuses are protected from destructive maternal immunity during pregnancy by an enzyme called IDO. IDO also has key roles in the other diseases mentioned above. Recently it has been shown that IDO causes pain, a problem in many clinical diseases. We want to understand how IDO activity protects healthy as well infected and cancerous cells and causes increased pain and then use this new knowledge to prevent or alleviate disease and reduce undesirable consequences of disease such as pain. We found new ways to modify IDO activity in mice and we will test these procedures using mouse models of human disease. Project goals are to find out how IDO impacts disease development and treatments. Five overlapping research objectives are integral to the project; (1) to understand how IDO is induced by inflammation and the effects of inducing IDO; and elucidate how IDO promotes (2) cancer development (3) and chronic infections but inhibits (4) autoimmune disease and (5) radiation sensitivity. In summary, our goals are to study what turns this enzyme on, how it protects cells from attack by immune cells and causes pain, and to use this new knowledge to develop better ways to treat patients and alleviate pain.
What are the potential benefits likely to derive from this project (how science	Potential benefits likely to accrue from the research are: 1) Increased knowledge of key molecular and cellular

could be advanced or humans or animals could benefit from the project)?	pathways that drive or impede diseases involving the immune system, that affect many people 2) Identifying new targets and reagents to prevent, treat or cure these diseases 3) Validation of new treatments to manipulate the immune system as a means to prevent, treat or cure disease in mouse models, as the initial step towards experimental clinical trials in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (~16,000 over 5 years)
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?	Adverse effects include weight loss, reduced immune functions and pain, which may manifest transiently or chronically. The severity of procedures to be applied will not need to exceed moderate level in most cases, and in many cases will be mild. In rare cases, severity levels may exceed moderate level to permit rigorous evaluation of the effectiveness of novel treatments given to other mice in the same experiment. All mice will be killed by a Schedule 1 method after experimental use.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune system is complex and is dispersed in the body. Many immune cell types of research interest cannot be cultured outside the body (*in vitro*) and small numbers of distinct immune cell types have pivotal effects on immune responses. Non-animal alternatives, such as *in vitro* cell culture cannot replicate the complexity of physiologic responses to defined challenges that incite (or inhibit) disease processes in organisms. Hence *in vitro* studies are severely limited in scope and can only be used to provide some mechanistic insights, which must be validated in organisms. Moreover, due to the propensity for diametric effects in immune responses it is imperative that insights from *in vitro* studies are evaluated carefully in organisms, as misleading (at best) or fatal (at worst) consequences may emerge if diametric responses manifest *in vitro* and *in vivo*.
2. Reduction	Hypotheses and experimental designs will be crafted to minimise the numbers of mice used and the

Explain how you will assure the use of minimum numbers of animals	duration of experimental studies. Statistical power analyses will be undertaken with expert advice from statisticians to ensure minimum use of mice to test particular scientific hypotheses posed.
3. 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.	Humans and mice are mammals and share many evolved biologic features, including remarkably similar immune systems. The mouse models we have selected share multiple features with clinical diseases in humans. In addition, many genetically altered (GA) mouse strains are already available for the research we propose, as well as many reagents needed to analyse and modulate immune responses that can be used in mice. General measures to minimize welfare costs are; 1. Group sizes, treatments and study durations will be limited to the minimum necessary to address research objectives using ARRIVE guidelines to ensure scientific rigour, as well as minimizing welfare costs. 2. Mice will be monitored regularly to identify distressed animals and supportive care will be provided to mice that exhibit adverse effects indicative of distress and suffering. Supportive care includes (but is not limited to) access to moist food, warmer environments and subcutaneous fluids. 3. Humane endpoints, adverse effects and control measures are described for each Protocol. Mice are killed humanely if these endpoints are reached.

Project 28	**Tregs in lymphopaenia associated autoimmunity**	
Key Words (max. 5 words)	Lymphopaenia, Autoimmunity, Immunotherapy	
Expected duration of the project (yrs)	5 years Lymphopaenia, Autoimmiunity, Immunotherapy	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims: 1) To understand, and identify ways of preventing the main side-effect of alemtuzumab, an exciting new drug for multiple sclerosis (MS). Alemtuzumab works by binding to and killing lymphocytes - cells of the Immune system which normally fight infection but which mistakenly attack the brain and spinal cord in people with MS. Alemluzumab is highly effective, however as the immune system grows back after treatment one in three patients develop a new autoimmune disease, that is their immune system begins to attack another part of their body (mainly the thyroid gland). We believe the problem lies with a particular type of cell called the ‘Treg”. In health, Tregs suppress the immune system, keeping potentially harmful cells in check. This project aims to test the Tregs after alemtuzumab — to understand why they are defective (if they are) and to work out ways in which Treg function could be improved.	

	2) To understand why, in a clinical trial of individual with MS being treated with alemtuzumab, a drug called Palifermin prevents production of new immune cells from the thymus (a gland in the chest that produces new cells). We want to understand our trial result because it is opposite to what Is seen in animal studies — where Palifermin increases cell production by the thymus.
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)?	If Tregs after alemtuzumab are confirmed to be defective, it follows that autoimmunity after alemtuzumab might be prevented by altering the way in which these cells grow back. Enabling patients to receive alemtuzumab without the risk of autoimmunity would be a huge step forward in the management of MS. It is also important for us to understand the effect of Palifermin on the human thymus, as multiple teams of researchers around the world are using! planning to use palifermin to increase human thymic function. In addition, this project will advance our understanding of how the human immune system works; it may also help people who develop similar immune-complications after chemotherapy, and following treatment of HIV.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, 5000 will be bred of which more than 2/3rds will be part of various studies, over the 5 year course of the licence.
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?	NA
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Autoimmune disease involves many interacting cells that have developed and migrated within the autoimmune prone environment and so the whole body

use non-animal alternatives	must be analysed to understand the progression of disease over time, Similarly, whole animal models are essential for the testing medication and no alternatives currently exist.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Group sizes for experiments are miriimised in consultation with our statistics group so as to make sure that we are using the minimum number of animals to answer our scientific question robustly. Most experiments are done on groups of 3-6 mice and experiments repeated up to 5 times to be confident of results. Depending on results obtained, group sizes may be adjusted but experimental protocols are not usually pursued if they require a group size of greater than 6.
3. 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.	Wherever possible we will aim to answer our scientific questions without using animals, for example by studying cells in culture dishes. However some questions can only be answered by studying animals. We have selected to study mice and in particular plan to study NSG mice. These mice do not have an immune system of their own, and so can be used to study the human immune system. Using these animals replaces to some extent the need to work on non-human primates. Throughout our work we will closely monitor our animals to ensure that harm is minimised. We will constantly review our work and will stop performing these experiments if we are not able to generate high quality, meaningful results. We will ensure that we remain up to date with the scientific literature and will seek to improve/refine our practices whenever possible.