Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 10

Projects with a primary purpose of: Translational and applied research – Human immune disorders

Project Titles and keywords

1. **Infection and Immunity**
    - Infection, immunology, vaccine

2. **Role of macrophages in Neuromyelitis Optica**
    - AQP4, monocyte/macrophage, mouse, NMO, NMO-IgG

3. **Immuno-modulatory and Inflammation Research**
    - autoimmune disease Immune system, modulation

4. **Allergic disease and immunomodulation by parasites**
    - Allergy, asthma, virus, parasite

5. **Regulation of Immunity and Immune-Mediated Disease**
    - Autoimmunity, Immunotherapy, Transfusion, Cancer, Inflammation

6. **Regulation of leukocyte recruitment during acute and chronic inflammation**
    - Leukocyte Recruitment, Rheumatoid arthritis, Adiponectin-PEPITEM axis, Mesenchymal Stem cells, Fibroblasts

Project 1	**Infection and Immunity**	
Key Words (max. 5 words)	Infection, immunology, vaccine	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
	X	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 purpose of this programme is to support the development of drugs and vaccines which can be used as treatments against infections and immune disorders for human and animal use.	
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)?	There are many infective diseases where there is, as yet, no reliable treatment or no cure (e.g. influenza). These illnesses can be debilitating and unpleasant for the sufferer and in some cases cause severe illness and be fatal. The testing under this project will help identify new vaccines and drugs which may be used as anti-infective agents or modify the immune system to protect people and animals against a wide range of infectious and immune disorders.	
What species and approximate numbers of animals do you expect to use over what period of time?	The project will last for 5 years-animal numbers as follows: Mouse 15000 Rat 5000	

	Guinea pig 500 Ferret 500 Hamster 250 Rabbit 250
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of studies will be conducted using rodents. Most of these studies will involve animals being dosed with test substances or vehicle and bled afterwards or killed humanely to provide tissue samples. The animals will usually show nothing more than minor discomfort from dosing and bleeding procedures In some studies animals will be infected with bacteria, viruses or fungi (for example) and will be expected to show symptoms of infectious disease. Most of these studies will be designed to produce an infection that will not cause intense symptoms. During these studies, infected animals will be regularly checked (including during the night) to monitor the animals health status. If the animals reach their humane end points they will be killed. If the scientific objectives of the study are met before this, then the animals will also be humanely killed. In some studies, it may be that to evaluate a new treatment properly, that more intense symptoms need to be produced in an animal to mimic the actual clinical situation a patient in hospital may experience. If this happens, animals will be checked more regularly (at least 4 times per day) and under a well set and defined criteria, if their disease becomes too intense, they will be humanely killed. This will be for a small number of animals overall. All animals used under this licence will be humanely killed at the end of procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The intact Immune system is a complex system which is not fully understood and therefore for the work under this project, there is no adequate model to replace the whole animal experimental model, as

use non-animal alternatives	the complex mechanisms under investigation cannot be adequately modelled in test tubes in the laboratory. Similarly, infection is a complex disease process involving the immune and inflammatory systems as well as the toxicity of the infective agent itself. It cannot be adequately modelled in a test tube as the host/agent interaction is too complex.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments will be designed in order to achieve the scientific objectives using the minimum numbers of animals. For study types that are less well established and for which historical data may not be available, published scientific literature will normally be consulted to help establish the group size. Statisticians are often consulted particularly where the study type is not routine. Where possible, common control groups will be used wherever possible in order to minimise the numbers of groups used. For less established study types, a preliminary phase or 'pilot study' may be conducted whereby smaller numbers of animals may be used to generate data in order to ensure that the experiment operates to expectations and to generate some data which may be used to optimise the study design.
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.	Rodents and guinea-pigs will be mainly used for the tests conducted under this licence. These species are considered to be of the lowest neurophysiological sensitivity available with achieving the study aims. The ferret may be used for discrete modelling of some infectious disease (e.g. influenza and other viral infections) due to its similarity in clinical signs, and immune and inflammatory responses to man. Generally, for infection models, a measurement of the microbiological agent in the organs will be used whenever practicable as this means the animal will get a clinically relevant infection without displaying intense symptoms of infection. The assessment would be made post mortem after a period of infection and after treatment with test materials

	designed to be anti-infective For all infection models signs of clinical disease will be carefully assessed (as a minimum) by observation, recording of clinical signs, body temperature and bodyweight 4 times a day (including during the night) in the first 48h after infection, with the frequency of observation thereafter being dependent on the clinical condition of the individual animals. If the clinical signs of infection are seen, supportive measures (e.g. extra bedding, more palatable food, food and water supplements) will be employed for animals exhibiting clinical signs, and the frequency of observation will be increased. The limits on clinical signs to which the animals are allowed to display are defined in this licence, and when they are reached, the animals will be humanely killed. For the management of animals undergoing surgery, an aseptic approach will be used. These surgeries will be carried out by appropriately trained and qualified persons. Appropriate analgesia will be provided as required. Surgical procedures will be carried out in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2010). In the event of post-operative complications, animals will be killed unless, in the opinion of the a vet, such complications can be remedied promptly and successfully using no more than minor interventions Care is taken to provide as much environmental enrichment as possible working parties that often tests and introduces new environmental enrichment are in place

Project 2	**Role of macrophages in Neuromyelitis Optica**	
Key Words (max. 5 words)	AQP4, monocyte/macrophage, mouse, NMO, NMO-IgG,	
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)	Neuromyelitis optica (NMO) is a severe disease that affects the brain. Most patients have a substance in their blood (antibody) termed NMO-IgG that kills brain cells called astrocytes. Current treatments are not very effective and have serious side effects. We developed a mouse model of NMO that involves injecting NMO-IgG in the brain. This mouse model closely mimics the type of brain damage we see in NMO patients. A key question is the role of blood cells called macrophages. In our NMO mouse model, macrophages begin to enter the brain at day five and persist for two weeks. Macrophages are the most abundant type of blood cell found in the brain of humans and mice with NMO. It is unknown if macrophages are harmful (by killing brain cells) or if they are beneficial (by getting rid of already dead cells). The role of the macrophage	

	subtypes M1 and M2 is also unclear. This project aims to clarify the role of macrophages in NMO. There are several Aims: **To find out if macrophages are beneficial or detrimental.** We will see whether eliminating macrophages reduces brain damage in NMO. **To compare the number of M1 and M2 macrophages in the NMO lesion.** We will find out the relative numbers of M1 and M2 macrophages in the brain at different times. Our prediction is that M1 macrophages are the first to enter followed by M2 macrophages. **To find out the roles of M1 and M2 macrophages.** Two experiments will be done: a) We will inject M1 macrophages in some mice and M2 macrophages in others. We will compare the amount of brain damage in our mouse NMO model. We predict worse outcome in mice that received M1 macrophages compared with mice that received M2 macrophages. b) We will give medicines to increase the number of M2 macrophages. We predict less brain damage in drug-treated mice compared with mice not receiving the drugs.
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)?	NMO is a devastating disease that affects the brain and can cause blindness, paralysis and death. It is thought to affect about 1 – 4 / 100,000 people, though the exact frequency is unknown. In addition to the morbidity of the disease, the cost to the health service is substantial, because many NMO patients are dependent and require chronic supportive care. Current NMO treatments are non-specific and have serious side effects by suppressing the immune system. Our understanding of how brain damage occurs in NMO is limited, which precludes the development of more effective and less harmful drug treatments. This project will provide important new information for researchers and will improve our understanding of

	how NMO causes brain damage. We predict that M1 macrophages are harmful and M2 macrophages are beneficial in NMO. This would be an important finding because it would implicate the macrophage as a key player in brain damage associated with NMO. We may, therefore, be able to treat NMO by using drugs that target macrophages. For example, If we find that M2 macrophages are beneficial in NMO, then medicines that increase the number of M2 macrophages may be novel treatments. We may also be able to use macrophage counts in the blood to predict when an NMO attack will occur. We predict that changes in the relative numbers of M1 and M2 macrophages in the brain and blood may be used to establish the age of NMO lesions or to predict recurrence.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 years we expect to use about 270 adult mice.
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 administration of NMO-IgG into the brain of our mouse NMO model does not cause distress in mice. Mice eat, drink and groom normally.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is impossible to mimic the complex structure of the brain and the complex interactions of the different brain cells and blood cells using non-animal alternatives. In some experiments we will use non-animal alternatives. For example, we will screen many drugs for their ability to increase the number of M2 macrophages using non-animal systems. Only the two most effective drugs will be tested in mice.
2. Reduction Explain how you will assure	We will use no more than 10 mice per group based on statistical calculations. Smaller effects that need

the use of minimum numbers of animals	more mice to prove are generally not clinically important. We will obtain many outcomes per mouse. Each mouse generates a lot of different types of information thus reducing the numbers of mice used. When testing potential new treatments we will screen the medicines using non-animal methods to ensure that only the most promising compounds progress to animal tests.
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 animal NMO model we developed has been characterised in mice. This model has been used extensively to study NMO. It is reproducible and widely validated. We, therefore, propose using this mouse model. Death from injection into the brain is rare. We minimised the chance of death by using a small injecting needle, minimising ~~reducing~~ the number of injection sites, reducing the injection volume and injecting only once per site. The NMO mouse model does not produce major behavioural abnormalities even with large brain lesions. After the injection, mice do not appear distressed.

Project 3	**Immuno-modulatory and Inflammation Research**	
Key Words (max. 5 words)	autoimmune disease Immune system, modulation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	Yes	Translational and applied research
	Yes	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)	Auto-immune diseases, such as rheumatoid arthritis, some cancers and inflammatory bowel disease are caused by the immune system attacking its own body. It is thought that common biological mechanism(s) cause these and other auto-immune diseases. New medicines are required because the treatment of patients with these types of diseases is currently unsatisfactory with only some symptoms being reduced but not permanently cured. The primary aim of this programme of work is to identify new treatments for human diseases. Identification of novel therapies which are effective in animal models of immuno-modulation and inflammation and identifying new pathways involved in immuno-modulation and inflammation diseases will lead to the development of medicines to treat such human diseases. Although assessment of potential new medicines in test-tubes will provide valuable information, we need to follow the effects in the whole animal to ensure	

	that the immune system is responding as predicted.
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 overall benefits of the work are to improve the understanding of auto-immune diseases and to develop improved medicines for patients with auto-immune diseases. Diseases such as Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Inflammatory Bowel Disease (IBD), Cancer and Psoriasis are major diseases within this category and affect a large proportion of the human population worldwide. RA affects between 0.5 and 1% of adults in the developed world. Cancers as a group account for approximately 13% of all deaths each year. These diseases not only affect the patients' lives but also those of their families and carers and potentially overload the health care systems. Currently there are treatments that may reduce some symptoms of these diseases in some small percentage of patients, but none work in all patients. Results from studies performed under this licence will provide key information on the likelihood of a particular new medicine working in these types of diseases
What species and approximate numbers of animals do you expect to use over what period of time?	Up to a maximum of 11,250 rats and 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?	We need to study parts of the immune system as it works in the whole animal. Some animals will have their immune system stimulated and the acute effects of this will be studied. In other studies we will induce states similar to early rheumatoid arthritis, cancer and inflammatory bowel disease (IBD). This may result in swelling of the limbs or production of cancerous lumps or symptoms of IBD such as weight loss, diarrhoea or bloody faeces in some animals. We will take blood samples, measure markers of the response and determine whether new medicines can affect the response. Some animals will experience discomfort. This will be monitored. Any animal displaying discomfort and distress beyond the minimum required to achieve the aims of the study (e.g. piloerection, hunched posture, subdued

	responsiveness, weight loss >20%) will be killed humanely. Anaesthetics and analgesics will be used to reduce the discomfort induced by any surgical procedure. The use of analgesics may impact the disease progression and interfere with the outcome of potential new medicines being tested. Carprofen is routinely administered prior to and for one day post surgery as an analgesic. This substance is a non-steroidal anti-inflammatory agent and hence its long term use may interfere with the biological systems involved in these studies and may inadvertently affect the study outcomes. Rats and mice with arthritis will display swelling of the limbs however, they are expected to still be able to move around their cage, interact with cage mates and display normal feeding and grooming behaviours. Should any animal not be able to display these behaviours it will be killed humanely.. The investigation of the new medicines should reduce any suffering experienced by the animals. At the end of studies the animals will be killed humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All work using animals will be preceded by studies using human and/or animal isolated blood, organs, tissues or cell lines. The immune systems of rodents and humans are very complex with many different parts working at different times and in different ways sometimes together sometimes alone. We can study parts of the immune system in test-tubes but cannot study the immune system as a whole, so we have to use animals with an immune system like humans. The immune system of rodents has been extensively studied, and is sufficiently similar to human to provide appropriate models for this work.
2. Reduction Explain how you will assure	The estimated number of animals is based on our previous experience of designing these types of studies. For new study designs we will consult with a

the use of minimum numbers of animals	statistician to ensure that we are using the minimum number of animals to achieve the objectives.
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 will read the scientific literature and document how the most appropriate design for the aim of our studies will be chosen. Challenges to the immune system, the volumes of injections and the number of blood samples taken will be balanced limited to ensure that the welfare of the animal is not compromised and that the level of discomfort is kept to a minimum, while achieving the scientific need for these studies. Animals will be routinely group housed with appropriate litter, nesting material and environmental enrichment. A veterinary specialist will be available to advise on care for the animals and can be contacted outside normal working hours if necessary.

Project 4	**Allergic disease and immunomodulation by parasites**	
Key Words (max. 5 words)	Allergy, asthma, virus, parasite.	
Expected duration of the project (yrs)		
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 overall aim of this project is to develop novel medicines from parasitic worms to treat human allergic diseases. We aim to show that parasite products can treat asthma (caused by airbourne allergens like pollen or lung viruses), eczema and food allergies. We will investigate how these diseases develop, and which molecules and mechanisms parasites use to suppress this. This information will allow us to develop new drugs for human allergic diseases.	
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 develop new medicines derived from parasitic worms to treat allergic diseases. It will investigate how the immune system malfunctions leading to disease, and how these aberrant responses can be controlled. This work may lead to the direct development of parasite molecules as new drugs for human diseases, or lead to the production of derivatives of these, or identify novel pathways which could be interfered with in order to affect	

	pathology. These pathways are shared between many allergic diseases (e.g. asthma, eczema and food allergy), and similar pathways take place in inflammatory diseases (such as inflammatory bowel disease), thus this work has the potential to benefit large numbers of people. Asthma affects 300 million people worldwide, 1/12 of the UK population, and kills 3 people a day in the UK. The NHS spends around £1 billion a year on treating asthma, 50% of this on severe and steroid-resistant asthma (although this only affects around 5% of asthmatic individuals), which this project is aimed at combatting. Thus asthma is a huge public health problem, and current treatment regimes are aimed only at combatting symptoms, not the route cause of the disease. Likewise, atopic dermatitis affects 20% of all children worldwide, and food allergy affects 250 million (Pawankar, 2013, WAO White book on Allergy). Thus treatments aimed at combatting these diseases have the potential to improve the lives of huge numbers of people, with knock-on economic and societal benefits, reducing treatment costs and work days lost to illness.
What species and approximate numbers of animals do you expect to use over what period of time?	Around 8,900 mice and 150 rats are expected to be used over the course of this 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?	Most animals will experience mild or moderate levels of severity. Parasitic infections generally do not cause significant pathology, and at the doses used here, infected animals appear identical to uninfected animals. Models of allergic asthma and respiratory virus infection necessarily cause some pathology in the form of inflammation in the lung, however this does not cause the animals great amounts of distress, and any which appear generally ill or have difficulty breathing will be immediately sacrificed. Models of atopic dermatitis likewise cause pathology in the form of scaling and thickening of the skin, however this should not cause the animals more than moderate discomfort, and again any mice which do

	show more severe pathology will be immediately sacrificed. At the end of the experiment, all 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	When modelling human diseases, the mechanisms that cause pathology are so complex, and incompletely understood, that animal models must be used: to use only experiments in the lab with individual molecules or cell types is to risk missing an important effect which is not present in the in vitro system used. Therefore, the majority of work in disease models will be done in animals, in order to characterise pathways of allergy and suppression thereof. However, once these pathways have been elucidated, experiments in the lab will be utilised test parasite-derived potential treatments. As an example of this, we recently found that parasite products suppress pathology in a mouse model of asthma: investigations of the immune pathways involved showed that this was through suppression of the release of an immune messenger in the lungs. In order to screen candidates, we then set up a simple lab assay using cells cultured with allergens to induce this messenger release and various candidate molecules. This allowed us to identify a messenger suppressive molecule without having to use large numbers of mice to test multiple candidates. Once the candidate was identified in the lab, we then showed that it worked in live mice, ensuring relevance of results. We will then aim to translate results to the human disease, we will move as rapidly as possible to experiments using human cells in the laboratory, to show these molecules are worth testing in human clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers	This work uses products of parasitic worms to suppress allergic disease. Previously, we have used parasite products collected from parasite cultures: these parasites themselves must be harvested from

of animals	infected animals. Thus, by identifying and synthesising individual immunosuppressive molecules from parasites, we have greatly reduced the need for animal-derived parasite material. In doing so, we have reduced the need for mice to maintain the parasite lifecycle at high levels, reducing mouse numbers from 50 a month for parasite production, to 20 (a minimum to maintain the parasite lifecycle). Further reductions in mouse numbers will be made by improvements in experimental design and statistical methods. Furthermore, by pooling human and laboratory resources within the research team, we can collect and assess far more information from the same experiment: this prevents us from having to set up multiple identical experiments to test different hypotheses. We have ready access to colleagues in the Institute with excellent statistical training and established meta-analysis methods for refining experimental protocols allowing us to aggregate data across multiple experiments, in the interests of reaching a statistical conclusion without conducting additional 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.	Mice are the species of choice for almost all the work envisaged in this Project, as their immune system is well-understood and manipulatable, and well-defined models for many human diseases have been characterised. The availability of transgenic (genetically-altered) mouse strains and commercially-available products aimed at investigating mouse immune responses provide an unrivalled access to experimental manipulation at the most refined level currently possible. The only experiments in which we will use another species, rats, are where we will use a parasite which is naturally more infective to rats than mice. Comparison between these two parasitic species will allow us to widen our conclusions and should aid application to humans.

	We will continue to use the least invasive and distressing procedures available, to reduce pain, suffering, distress and lasting harm to experimental animals. An example of this is administration of substances to the airways: previously, intratracheal administration (involving introducing a tube down the airway of an anaesthetised animal) was used which caused some inflammation in the airways, presumably with some discomfort for the animal. In all experiments involving airway administration, we have now changed to using intranasal administration: this technique uses very rapid inhalational anaesthesia, followed by administration of substances to the nostrils, and inhalation through normal respiration. This process takes less than 1 minute, is easy to master, and results in mice which have recovered fully within 5 minutes. We have also optimised protocols for other systemic sensitisation protocols, by reducing numbers of individual procedures and total length of experiment: previously we used a 31-day protocol to model asthma using 2 injections, but a 17 day procedure using a single injection was found to be equally effective. We will at all times conform to guidelines for animal housing, including environmental enrichment and acclimatisation where mice have been moved from other locations. Mice will always be housed in groups in the experiments described – no individual housing will be required.

Project 5	**Regulation of Immunity and Immune-Mediated Disease**	
Key Words (max. 5 words)	Autoimmunity; Immunotherapy; Transfusion; Cancer; Inflammation	
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)	The work described in this licence arose directly from the studies of clinical problems faced daily by patients with severe immune-mediated or inflammatory disease, including transfusion reactions, and cancer. The immune system evolved to defend us against infections, but immune-mediated disease occurs when its ability to distinguish “self” or “harmless” from “foreign” or “dangerous” fails and it mistakenly attacks the wrong targets to cause injury. These diseases are common (affecting up to 25% of the population at some stage of life) and contribute significantly to some of the most devastating chronic illnesses that affect the developed world, including arthritis, diabetes and kidney failure. They also include allergies and reactions to blood groups and grafts. Current treatments are unsatisfactory and typically consist of drugs that suppress the whole immune system. Not only are these relatively ineffective but they can also be dangerous - not least through	

	increased susceptibility to infection. There is a clear need for better treatments for these disorders that are both potent and targeted precisely to switch off only the harmful immune responses, but this requires deeper understanding of the reasons why control the immune system fails and tissues are attacked. Understanding how to control immune responses will also lead to treatments that boost immunity where this is lacking, for example in cancer. Our work aims to understand, and then develop treatments based on, the balances between harmful and protective immune cells, particularly the different varieties of cells called lymphocytes and macrophages.
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 strategy for our work is focused on two major steps. First, it is important to understand the basic mechanisms that control immunity, in order rationally to design novel approaches for therapies that selectively control damaging immune or inflammatory responses and stimulate immunity where it is required. Secondly, we will exploit the understanding gained to develop new, more targeted, treatments than those currently available for particular diseases where there is immune-mediated damage to circulating blood cells, or the kidney, or failure to make immune responses to cancers such as melanoma.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the next five years we expect to use up to 2000 mice and 130 rats.
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 vast majority of the work (95%) is expected to result in only mild adverse effects arising from selective use of animals with mild genetic alterations; administration, including by injection, of substances with benign profiles; routine blood sampling; and imaging under general anaesthesia. Studies of disease processes that attack circulating cells are designed to reproduce the relevant parts of the underlying responses without affecting wellbeing. In a small proportion of other animals, inflammatory disease affecting kidneys or other organs will be

	induced, but the signs will be mild or moderate with appropriate care. All animals are euthanased humanely at the end of each experiment for sample analysis, with much of the work on immune responses and inflammation carried out on cultured cells.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The animal studies will complement other parts of our programme of work in which isolated cells from human patients and healthy donors are assayed, and observational studies of human subjects. These human projects will continue, but the use of animals is essential because the immune system is too complex either to be modelled using cultured cells, or for predictions of the benefits of new treatments to be made solely from such approaches. Most (90%) of our work is carried out on cultured cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers are kept to the minimum required for statistical significance, with power calculations used where possible to predict how many are needed. GA lines which are no longer required will be frozen down.
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.	Careful choice of mouse and rat models, and the use of advanced techniques, means that the processes that cause disease can be studied, and new treatments tested, with the minimum effect on animals. Wherever possible, which includes the vast majority of experiments, the answers we need can be obtained without causing disease and require only minor interventions such as injections of benign substances and blood sampling. The animal studies are carried out in a purpose built facility with experienced animal care staff and a veterinary surgeon is on site the vast majority of the time.

Project 6	**Regulation of leukocyte recruitment during acute and chronic inflammation**
Key Words (max. 5 words)	Leukocyte Recruitment, Rheumatoid arthritis, Adiponectin-PEPITEM axis, Mesenchymal Stem cells, Fibroblasts
Expected duration of the project (yrs)	5 year(s) 0 month(s)

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

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;
No	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being	Inflammation is a protective response to tissue injury and infection. White blood cells (immune cells) recruited during the initial response need to be removed from the tissue to allow the response to

addressed)	stop. Errors that prevent inflammation from being stopped occur in a number of chronic inflammatory disease (e.g. rheumatoid arthritis [RA]) leading to long term tissue damage. Here we will look at how immune cell derived agents (e.g. PEPITEM) and tissue-resident cells (e.g. fibroblasts or mesenchymal stem cells) control the movement of immune cells in health and disease. Initially we will study these using laboratory based models incorporating human cells from healthy subjects and patients with different types of inflammatory arthritis. We will confirm these findings using animal models of health and arthritis to address the 3 following questions: Is immune cell movement changed by: 1) Treating mice with adiponectin-PEPITEM axis modifying drugs? 2) Treating mice with cell therapy (e.g. MSC)? Tissue-resident cells (fibroblasts) from patients with acute resolving or chronic persistent inflammation?
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 advance scientific knowledge surrounding what controls the movement of immune cells from the blood, into and through the tissue during inflammation and how this goes wrong in disease. It will highlight to what extent PEPITEM, mesenchymal stem cells, or fibroblasts control these processes and whether these offer new therapeutic targets and/or agents
What types and approximate numbers of animals do you expect to use and over what period of time?	Mice Approximately 800 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 levels of severity? What will	Mice will get moderate inflammation within the joints of the front and back limbs. When pain becomes apparent in these animals they will be administered with routine analgesics to manage this. Before pain and inflammation pass a moderate level, animals will

happen to the animals at the end?	be killed to prevent any on-going discomfort. Certain models of arthritis in the mice will require between 1 and 3 injections containing reagents that induce arthritis. These will cause mild discomfort and animals will be monitored closely throughout procedures and killed where adverse effects are identified to prevent any on-going discomfort. Some animals will undergo surgery for tissue/pump implantation. Pain will be pre-emptively managed with routine analgesics to manage this. Before pain and inflammation pass a moderate level, animals will be killed to prevent any on-going discomfort. We have taken precautions to reduce any suffering from other procedures including anaesthesia, therapeutic agents, and blood withdrawals by keeping the number of these procedures to a minimum.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	Murine animal models are highly effective in driving our understanding of the pathophysiology of inflammatory disease. They allow us to perform in vivo studies that would be otherwise physically or ethically impossible in human cohorts and provide a translational link between basic and clinical research. In this study, murine models are essential to address the objectives outlined within this proposal. First and foremost, only in these animal models of the disease can we determine whether influencing immune cell recruitment will protect against clinical symptoms of arthritis. Essentially, this will provide the validation and rationale to examine this in human inflammatory disease such as RA.
2. Reduction Explain how you will ensure the use of minimum numbers of animals	A key strength of our work is that it combines both human and animal models so that each can be used to inform the other and therefore minimize an over reliance on mouse models of disease. Carefully refined statistical analysis will ensure that we use the least number of animals to provide a

	meaningful answer to our research questions. Pilot studies throughout using small experimental numbers will ensure that no large experiments will go ahead unless they will provide meaningful results. Using non-invasive strategies such as imaging of joints or other tissues in the same animal over time will also reduce the number of animals required allowing statistically significant differences to be obtained from less mice.
3. Refinement Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the best model for the study of persistent disease because: • The main components of their immune system is shared by humans; this is essential where immune responses as opposed to the function of individual genes is being studied and thus will produce satisfactory results • An extensive range of reagents is available for analysis of immune responses • They are the most acceptable animal model that shows the least degree of neurophysiological sensitivity and will suffer the least pain, suffering, distress, or lasting harm. • There are no other alternatives to this work. We are employing models that have been refined and streamlined as much as possible by our collaborators at another establishment.