Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 23

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

Project Titles and keywords

1. **Provision of Blood, Tissues and Antisera**
    - Provision of Biological Materials

2. **Stem Cells for Blood and Tissue Repair**
    - Blood, blood vessel, repair, transplantation, regeneration

3. **Kinetics, dynamics & diagnostics of CB agents & antidotes**
    - Pharmacokinetic; pharmacodynamic; toxicokinetic; toxicodynamic, antidotes

4. **Repopulation of the blood system in zebrafish**
    - Haematopoiesis, haematopoietic stem cells (HSCs), zebrafish

5. **Restoring Control of Immune Responses in the Retina**
    - Retina, immune, responses, restoring, control

6. **Drug and vaccine formulation and delivery**
    - Drug delivery, vaccines, formulation

7. **Novel vaccine platforms targeting chronic disease**
    - Vaccination, chronic disease, VLPs, immunotherapy

8. **Immune responses to persistent herpesviral infection**
    - Memory inflation, cytomegalovirus, adenoviruses, vaccine

9. **The immune response in wound healing and cancer**
    - Inflammation; wound healing; cancer; mice; zebrafish

10. **The Causes and Treatment of Autoimmune Diabetes**
    - Diabetes, insulin, immune system

11. **Intrinsic & extrinsic effects on B cell responses**
    - B cells, vaccination, mice, antibody

12. **Biology of carbohydrate-binding proteins**
    - Immunology, cell biology, glycobiology

13. **Parameters of macrophage origin and function**
    - Macrophage, ontogeny, stem cell, inflammation

14. **Immunology of cancer and pregnancy**

- Immunity, pregnancy, cancer, transgenes, mice

15. Mechanisms of T cell survival and homeostasis

- T lymphocyte, homeostasis, cancer, mice

16. Physiological regulation of innate immune responses

- Myeloid cell, Inflammation, Hypoxia, Metabolism, Mice

17. Molecular regulation of the immune response

- Inflammation, Cancer, Viral infection, Immunology

18. Regulation of immunity, inflammation and haematopoiesis

- Immunity, asthma, infection, cytokines, haematopoiesis

19. Mouse antibodies for biomedical research

- Immunisation mouse antibody adjuvant

20. Finding curative therapies for blood cancers

- Blood, haematology, stem cells, blood cancer

21. Blocking autoimmunity by targeting CD4 T cells

- Autoimmunity, CD4 T cells, Tregs, OX40

22. Phagocyte function and inflammatory bowel disease study

- Bacteria, Crohn's disease, neutrophil, granulomatous

23. EGFR mediated modulation of immune responses

- inflammation, immune regulation, tissue remodelling, protection against pathogens and wound repair

24. Modulators of Immunity and Inflammation

- Infection, therapy, autoimmunity, allergy, cancer

25. The role of B cells and microbiota in autoimmunity

- Autoimmunity, B cells, gut-bacteria

26. Evaluating Candidate HIV/AIDS Vaccine Strategies

- HIV, Vaccines, Pathogenesis

27. Molecular Networks of Inflammation

- Immunology, Inflammation, Autoimmunity, Signalling

28. Production of Blood Products

- Animal infection antisera

29. Pathophysiology of autoimmune diseases

- Autoimmune disease, immune molecules

30. Investigating the immune response to somatic corneal stem cell allografts

- Cornea, stem cell, immune system, transplantation, graft rejection

31. Impacts of urban development on avian immune systems and disease

- Immune response; blackbirds; *Turdus merula*; urbanisation

32. An ecological approach to infection and immunity

- Ecology, coinfection, immunity, helminths, wild mice

33. Initiation, resolution and modulation of inflammation

- Autoimmunity; allergy; inflammation; T cells; therapy

34. Type 2 immunity in infection and tissue homeostasis

- Helminth infection, cytokines, macrophages, wound repair, allergy

35. Does steroid production in inflammatory arthritis cause joint destruction, muscle wasting and bone loss?

- Rheumatoid arthritis, Steroid metabolism, Bone loss, Muscle wasting, Cartilage erosion

36. Polarised secretion from lymphocytes

- Immune cells, cancer, viral infections

37. Neonatal bacterial meningitis – infection and treatment

- *In vivo* imaging; *Escherichia coli* K1; blood-brain barrier; Group B streptococcus; neonatal bacterial meningitis

38. Immune responses promoting inflammatory disorders

- Innate immunity, cancer , inflammation, imaging

39. Production of *Hymenolepis diminuta* ova in rats

- Hymenolepis diminuta ova rat tapeworm

40. Drug assessment in models of multiple sclerosis

- Multiple sclerosis, therapy, immunology, demyelination, remyelination

41. Enhancing the value of selective breeding for parasite resistance

- Parasitology, livestock, genetics, immunology, pathology

42. Pathogenesis mechanisms of bacterial pathogens

- Macrophages, innate immunity, *Listeria*, *Streptococci*, *Staphylococci*

43. The Production of Antibodies

- Antibody, Polyclonal, Monoclonal, Immunogen, Antigen

44. Mechanisms of lymphocyte activation

- Lymphocyte, immunoresponse

45. The immune system as gateway for mental and physical wellbeing

- Emotion, Immune-response, translational medicine

46. Mice with human immune systems for drug discovery

- Human immunoglobulins, vaccines, mice, T-cell-receptor

47. Regulation of the Immune Response in Tissues

- Immune response, infection, cellular immunotherapy

48. Inflammation and Immunity in Digestive Diseases

- Inflammation liver gut pancreas

49. The role of circadian clocks in immunity

- Circadian, Inflammation, Lung, Glucocorticoids, Parasites

50. Immune responses in Helicobacter infections

- Immune, responses, Helicobacter, infections

51. Perturbations of adaptive immunity in inflammatory arthritis

- Immune response, arthritis, cell migration, inflammation

52. Regulation of immune responses in health and disease

- Inflammation, cytokines, infection, repair, autoimmunity

53. Neutrophil function in infection and disease

- Inflammation, cytokines, infection, autoimmunity, neutrophils

54. NF-κB and MAP kinase signalling in immunity and cancer

- Autoimmunity; inflammation; cancer; mechanisms; therapy

55. The Production of Antibodies

- Antibody, Polyclonal, Monoclonal, Immunogen, Antigen

56. Understanding how vaccines work

- Vaccine, Infection, Natural Killer (NK) cells, T-cells, Ageing

<table>
<tr><td>Project 1</td><td colspan="3">Provision of Blood, Tissues and Antisera</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Provision of Biological Materials</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">This project licence enables biological materials to be provided from animals, for example blood, other tissues and antibodies. The biological materials will be used for detection, diagnosis, research or therapeutic purposes.</td></tr>
<tr><td>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)?</td><td colspan="3">The benefit of this licence is to ensure that biological materials to support a research request are available when required. Examples of research requests that would be met with the biological materials are:
Research carried out with the aim of preserving human life, following attack with Biological or Chemical agents. Rapid availability of biological materials in this case supports research to preserve human life and address human suffering as quickly as possible.
To conduct in vitro research with animal tissues, replacing experiments on living animals.</td></tr>
<tr><td>What species and approximate numbers of</td><td colspan="3">Species permitted under this licence are mice, rats, hamsters, guinea-pigs, rabbits, chickens, sheep,</td></tr>
</table>

animals do you expect to use over what period of time?	goats, pigs, marmosets and macaques. Over a 5 year period the licence permits a approximately 1700 mice, 1900 rats, 10 hamsters, 1800 guinea pigs, 500 rabbits, 70 chickens, 300 sheep, 40 goats, 40 pigs, 220 marmosets, 110 macaques. This is a service licence. Animals will only be used if a research request is received and considered justifiable. If no, or fewer, research requests for the species occur during the 5 years, no animals, or fewer animals, of that species will 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?	Where possible the biological materials will be collected from animals using non-recovery anaesthesia. In this case animals will experience transient distress due to restraint and transient discomfort from needle insertion if anaesthesia is given by injection. After that there are no other anticipated adverse effects as this is a non-recovery procedure, tissues are collected and the animal will be killed without recovering consciousness. The licence permits animals to have blood samples collected whilst conscious as a recovery procedure. The expected severity of a single blood sample will be mild. The licence permits re-use animals where possible to reduce the overall numbers used. An animal may be re-used for further blood samples on more than one occasion. If an animal reaches a cumulative severity of moderate owing to repeated mild severity blood samples, any further use will be for a single non-recovery procedure. Animals used for antibody production will be given an antigen, usually by injection and will have blood samples collected. Animals may experience a reaction to the antigen or adjuvant used with the antigen to promote a good antibody response. Typically this is a local reaction, for example a lump or scab at the injection site. The expected severity for antibody production is moderate. After use on this licence, animals will be kept alive for potential re-use, killed by a Schedule 1 method or killed by a non-Schedule 1 method.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This licence will only be used to provide biological materials from animals where no other alternative will suffice. All possible alternatives will be assessed for each individual request submitted prior to the request proceeding to use of animals. An example justification for use of live animals is for production of certain antibodies for which a whole immune system from a living animal is required. Tissues collection under this licence will replace live animal experiments in many cases, for example the licence permits non-recovery collection of brain slices to be used instead of live animals for electrophysiology investigations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This licence allows use of ex-breeding stock animals for collection of biological materials. For example if materials are collected from an ex-breeder at the end of its breeding life under non-recovery anaesthesia, this reduces the need to breed a new stock animal for tissue collection. Where possible and/or practical, multiple samples will be collected from each individual animal when used for non-recovery tissue collection. This will ensure maximum use of each individual reducing the need to use other animals. Choice of animal species will be used where possible to reduce animal numbers. For example antibody production in a small number of chickens replaces the need to use greater numbers of rodents or rabbits to produce the same antibody.
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 species of animals used on this licence will be selected in each case to represent the best animal models for the purpose with the greatest likelihood of achieving the objectives of the research request. Individual requests for biological materials will be reviewed to ensure the 3R's have been addressed to encompass best practice, for example requests for blood samples will be expected to follow best practice on blood sampling set out by the NC3Rs. The review process will ensure that the mechanisms to reduce adverse effects are incorporated. Adverse effects occurring for each research request will be recorded with further refinements to reduce adverse effects implemented as required.

Project 2	Stem Cells for Blood and Tissue Repair	
Key Words (max. 5 words)	Blood, blood vessel, repair, transplantation, regeneration	
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)	Blood stem cells are critical to life. These stem cells reside in the bone marrow where they can generate all blood cells over a lifetime. Disorders of these stem cells (e.g. leukaemias; sickle cell disease) reduce patients' quality of life and survival. Transplantation of blood stem cells, with or without replacing defective genes, is potentially curative. While 2013 marked the one millionth such transplant, this therapy is associated with significant morbidity/mortality resulting from chemo-/radio-therapy or graft quality. Our first aim is to increase the quality of blood stem cells in the graft ex vivo to improve their take in the bone marrow and hence reduce associated morbidity and mortality. This will be accomplished i) by expanding stem cell numbers with specific factors in vitro before transplantation studies in animals, ii) by repairing damaged to bone marrow to enhance blood cell production, and iii) to eradicate malignant disease or repair the defective blood stem cells. In this way, we will provide better treatments and hopefully cures for blood disorders. Many patients die from organ failure or from debilitating organ diseases. Since most organs require a blood supply for their survival and function, our second aim is to regenerate a blood supply in	

	damaged tissues from stem cells using knowledge acquired in aim 1 in order to reduce the need for organ transplantation.
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)?	Blood cancers are the 5th most common cancer in the UK, and severe anaemias the most common inherited diseases worldwide. Over 1 million patients have received bone marrow transplants to treat these diseases, but, although successful, there is significant mortality and morbidity. Many millions of individuals suffer from diseases affecting the blood supply to organs (e.g. cardiovascular disease, stroke, chronic skin ulcers). The latter for example represent a silent epidemic affecting 1% of EU individuals. For diabetic patients, this can lead to limb amputation. In the UK chronic skin ulcers cost 3% of the NHS budget (>£3bn p.a.). Our aims are to further improve stem cell therapies for severe blood and organ disorders. While we will perform as many experiments as possible ex vivo, longer term stem cell therapies can only be tested for their efficacy in in vivo models ahead of clinical trials in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We would use a variety of mouse strains, particularly those that lack a functional immune system so that human cells are not rejected. The mice may alternatively be deficient in particular genes so that we can assess the function of these genes and their contribution to blood formation and tissue revascularisation and repair. Over a 5 year period, we would expect to use up to 3000 mice for breeding, a proportion of which would be used in assessing blood and tissue repair and regeneration, together with an additional 1500 for blood stem cell transplants, 500 for humanised niche studies and 500 for wound repair.
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?	1. Animals produced by crossbreeding may have an unexpected phenotype that could have an impact on animal welfare (e.g. less resistance to infections). 2. Myeloablation is used in human patients to reduce disease burden (e.g. in leukaemic patients) and/or to allow blood stem cell transplantation to occur. Any irradiated animals or animals receiving chemotherapeutic agents for myeloablation may show signs of adverse effects to radiation/chemotherapeutic agent or signs of delayed blood cell recovery (particularly up to 21 days post-transplant) such as lack of appetite, weight loss,

	listlessness and increased susceptibility to infection. 3. Genetically altered animals may display less tolerance to radiation or chemotherapy than wild type animals. 4. Animals administered with leukaemic/diseased cells may show signs of disease burden, and this will be closely monitored and controlled for. Treatments (e.g. analgesia, antibiotics, anaesthesia, heat therapy) will be administered where appropriate to minimise adverse effects. Monitoring procedures are designed to ensure that adverse effects are picked up early and appropriate actions taken to care for and ensure animal welfare. The level of severity is expected to be mild to moderate. At the end of the experiments, animals used on breeding or experimental protocols will be killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The repair using stem cells or their products of the damaged bone marrow, the long term generation of blood cells and the generation of a functional blood supply which contributes to the repair of organs or tissues can only be assessed by in vivo studies because interactions in different systems are needed to accurately predict successful clinical translation of new therapies or other.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will minimise animal numbers by careful cell/animal selection, by identifying statistically significant changes in cell functions in in vitro experiments, through careful experimental design, and by the use of human cell sources wherever possible before testing *in vivo.* Small pilot in vivo studies may be conducted first (e.g. to assess cell transplant numbers, to develop a humanised niche, to test irradiation protocols). To avoid culling animals at suboptimal time points, we may i) take small blood samples regularly to monitor stem cell take and ii) use live animal imaging to monitor outcomes in the same animal over time. Previous experiments or published data will be used to define animal numbers likely to give statistical significance, otherwise power calculations will be performed. By consulting a statistician before/during the experimentation, we will minimise/optimise animal numbers.
3. Refinement	Mice have the lowest neurophysiologic sensitivity to produce scientifically robust results and enable

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.	comparison with established outcomes. Suitable immunocompromised mouse strains are needed in experiments to test and prevent rejection of human materials as this increases the translatability of results. Enhanced human blood cell formation has been achieved with improved animal models (e.g. NSG mice), and we will use these or newer strains where possible. Radiation dosages depend on mouse strain used and its method of application. The lowest doses for effective transplantation of specific mouse strains will be used and administered as a split dose to reduce welfare impacts. We will use the husbandry method of adding baby milk power/mash to the cage and providing antibiotics before and following irradiation and house our animals in a ‘quiet room’ with minimal disturbance following transplantation to improve overall conditions for the animals and support the animals’ recovery and minimise harms. Surgical techniques for transplanting cells in scaffolds and live imaging will be carried out under general anaesthesia. Mice will receive pre-emptive analgesia to prevent pain. Where possible, we will use mice which do not have abnormalities which could compromise welfare when housed correctly. Wounds will be dressed to prevent damage by self-grooming and infection.

<table>
<tr><td>Project 3</td><td colspan="3">Kinetics, dynamics & diagnostics of CB agents & antidotes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Pharmacokinetic; pharmacodynamic; toxicokinetic; toxicodynamic, antidotes</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td rowspan="9">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td>✓</td><td colspan="2">Basic research</td></tr>
<tr><td>✓</td><td colspan="2">Translational and applied research</td></tr>
<tr><td></td><td colspan="2">Regulatory use and routine production</td></tr>
<tr><td></td><td colspan="2">Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td colspan="2">Preservation of species</td></tr>
<tr><td></td><td colspan="2">Higher education or training</td></tr>
<tr><td></td><td colspan="2">Forensic enquiries</td></tr>
<tr><td></td><td colspan="2">Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">A. To determine the behaviour of chemical warfare agents in animals as well as the behaviour of the antidotes against chemical and biological agents in animals.
B. To validate the ability of commercially available and novel methodologies to diagnose exposure to CB agents.</td></tr>
<tr><td>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)?</td><td colspan="3">Information on how antidotes to chemical and biological warfare agents behave in animals, which will be used to predict how these antidotes and agents will behave in humans. Also, data to validate computer models for the same purpose.

Testing diagnostic technologies to determine if they can indicate whether a person has been exposed to biological or chemical warfare agents.

Determining the correct doses of veterinary drugs for use in laboratory animal species.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Mice 1,500 Over 5 years

Rats 4,200 Over 5 years</td></tr>
</table>

	Guinea pig	300	Over 5 years
	Pigs/mini-pigs	250	Over 5 years
	Marmosets	100	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?	Animals may experience moderate transient pain following surgical procedures but this will be controlled through the use of pain relief. Animals will experience transient mild harm, distress or pain following administration of antidotes and subsequent Animals will experience transient mild pain following repeat collection of blood samples. If animals have been surgically cannulated they will not experience this transient mild harm, distress or pain as the necessity for restraint during sample collection is removed. Some animals will experience severe harm, distress or pain following exposure to large doses of chemical or biological warfare agents. These adverse effects may be controlled by the use of antidotes, unless the use of such antidotes will impact the scientific aim of the study i.e. determining the behaviour of the warfare agents in animals. The majority of animals will be euthanised at the end of procedures, however, some animals will be kept alive at the establishment and will be re-used in other studies. The suitability of these animals will be assessed by a veterinary surgeon and will only be re-used if the animal has experienced moderate severity harm, distress or pain, or less and general state of health and wellbeing has been restored.	
Application of the 3Rs			
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not ethically possible to carry out human clinical trials of chemical or biological warfare agent antidotes. Therefore, data from animals is critical. Determining how both the antidotes and the warfare agents behave in animals provides information to predict how they will behave in humans. Computer models exist for chemical warfare agents but not the antidotes. To build and test these computer models data from animal experiments are required. The research and development of antidotes against chemical and biological warfare agents will be reliant on animal data for the foreseeable future.		

	However, computer modelling will be used whenever possible.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The information generated by the studies detailed here will be used to inform the use of antidotes in separate studies that aim to determine the most effective way to administer antidotes. The maximum amount of information will be gained from each individual animal, reducing the requirement for more animals. All studies will be designed with input from a statistician to ensure the correct number of animals are being used to meet the defined objective. If appropriate, pilot studies will be carried out using a small number of animals: the information from these animals will be used to design subsequent studies. All relevant information from previous studies will be used to design the studies proposed here.
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 choice of animal species detailed here are those species used in other studies to show the antidotes are effective. The lowest possible doses of chemical or biological warfare agent or antidotes will be used in the studies, or where information exists human equivalent doses will be used. The purpose of these studies is not to determine how effective antidotes are, but to determine how they behave in animals. Animals that are unwell will not provide the information required, therefore, low doses will be used to minimise adverse effects. Diagnosis of exposure to chemical or biological warfare agents is critical in making sure the correct antidote is given. Diagnostic technologies must be assessed to make sure they work properly. This is first done without using live animals. However, the chemical or biological warfare agent may behave differently in living animals, therefore, the ability of a technology to indicate exposure must be tested with samples from living animals.

Project 4	**Repopulation of the blood system in zebrafish**	
Key Words (max. 5 words)	Haematopoiesis, haematopoietic stem cells (HSCs), zebrafish	
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)	Blood stem cells (BSCs) are immature cells of our bone marrow that generate trillions of new blood cells every day. BSCs can also restart the blood system in organisms that have lost the ability to make blood cells, for example patients that have undergone chemotherapy. This ability has turned BSC transplantations into the most common type of stem cell therapy. Yet, problems remain. Incomplete matching of donor and recipient blood cells can lead to transplant rejection and graft-versus-host (GvH) disease. While the former leads to BM failure, the latter causes side effects that can result in organ failure. A third of all patients do not to have a matched BM donor. Even those patients who receive a matched BM transplant frequently suffer from GvH disease (80% of all patients). Another unresolved issue concerns the number of transplanted BSCs. BSCs are rare and their number is limited in transplants. The smaller their number, the longer it takes for the patient's bone marrow to restart blood formation, leaving patients vulnerable to infectious diseases. Despite enormous efforts, we have not managed to maintain BSCs in culture, let alone increase their number. To overcome these problems, scientists are trying to generate BSCs in culture from other, more abundant cell types. Two approaches are currently	

	investigated. One reprograms cells into BSCs by forcing the expression of BSC transcription factors (TFs). The correct combination of BSC TFs will drive all BSC-specific genes and turn the cells into proper BSCs. The other approach tries to gently coach the cells by giving them signals. The right signals given in the correct order and at the appropriate time will guide the cells in a process that mimics embryonic BSC development. Progress has been made using both approaches, but remaining obstacles can only be overcome if we learn more about the signals and the TFs that control BSC development in the vertebrate embryo. To test whether particular genes are involved in BSC formation we need to be able to (a) manipulate the expression of the TFs and control the activity of signalling pathways, and (b) unambiguously identify BSCs in a model organism. This project licence is about establishing a reliable BSC assay in zebrafish. Because BSCs cannot reliably be identified by surface markers, they have to be tested functionally for their ability to restart the blood system and to maintain it long-term. Such long-term repopulation (LTR) assays have successfully been used in the mouse. Here we want to establish and use such an assay in zebrafish.
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 reliable zebrafish LTR assays will allow us to test wild-type and mutant transgenic blood cells for BSC activity, giving us insights into the roles of TFs and signalling molecules in BSC development. This will establish the zebrafish as an alternative to using mouse models for BSC testing. The proposed LTR assays are improved versions of a recently publish assay. The improvements refine the assay and are likely to reduce animal suffering. In the long-term, the gained knowledge will improve our chances to generate BSCs from more abundant cell types, and open up the possibility to provide patients with antigen-matched BSCs for transplantation therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	Zebrafish Danio rerio; about 760 animals in 5 years
In the context of what you propose to do to the animals,	Cells shall be transplanted into *cmyb* mutant Casper zebrafish. These mutants form normal numbers of

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	embryonic erythrocytes, but become progressively bloodless as the adult wave of BSCs fails to take over blood formation. In transparent Casper fish, lack of blood formation is easily recognised by the missing red colouration of the heart and the kidney. Without intervention, cmyb mutant fish would die from 11 weeks on. In the juvenile LTR assay, recipients will be anaesthetised and injected with cells into the vascular sinus behind the eye (retro-orbital) at about week 7 of development (weeks 5-9). Transplanted BSCs will rescue blood formation and adult development in the *cmyb* mutant. Fish in which blood formation does not recover will be culled in week 10 (moderate), i.e. before they develop severe symptoms. Rescued fish are allowed to grow to adulthood. They may be culled at a later stage to examine blood formation in the kidney marrow, the site of adult blood formation in the fish. Transplantations can cause tissue damage (in ~17% of the mutant fish) and can lead to infections (<5%). Similarly frequent are problems caused by the general anaesthesia (<5%). Animals that suffer from persistent infections or fail to fully recover from anaesthesia and injection will be culled to minimise their suffering (moderate). If BSCs can be enriched in smaller subpopulations, transplantations into pre-feeding stage embryos become feasible. These experiments will be done under anaesthesia. Occasionally, embryos may not recover from anaesthesia (<5%) or suffer from tissue damage (<5%). Injected embryos will be checked on days 4 or 5 and only embryos that are mildly affected will be allowed to grow up (mild).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	BSCs can neither be generated nor maintained in culture. The LTR assay in vertebrate model organisms is the only way to test them. There are no non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will carefully monitor our success rates and use appropriate statistical tests to ensure that the number of animals used is as low as possible, but sufficiently high to ensure statistical significance.
3. Refinement Explain the choice of species and why the animal model(s)	The optical clarity and the genetic amenability make zebrafish a superb model for elucidating the molecular details of BSC formation in the vertebrate

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.	embryo, allowing us to answer questions not easily addressed in the mouse. Fish are neurophysiologically less sensitive than mice. Our experiments use the latest zebrafish LTR assay. Firstly, zebrafish *cmyb* mutants possess no BSCs, eliminating the need to irradiate recipients to deplete resident BSCs. This will increase success rates and eliminates irradiation-induced welfare issues. Secondly, the use of Casper fish will allow early identification of fish whose blood system is not rescued. These can be culled humanely before they begin to suffer severely. Thirdly, establishment of LTR assays at pre-feeding embryonic stages of development will help us to avoid complications associated with the retro-orbital injection of juveniles and allow us to check blood system repopulation earlier.

Project 5	**Restoring Control of Immune Responses in the Retina**	
Key Words (max. 5 words)	Retina, immune, responses, restoring, control	
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 overall objective of the proposed research is to determine and understand mechanisms that cause dysregulated activation of the immune system in the eye, and drives tissue damage observed in various blinding diseases (e.g. Age-related Macular Degeneration [AMD], Diabetic retinopathy [DR], uveitis [intraocular inflammation]). Understanding the movement and dynamics of different immune cell populations responsible for damage to the eye will give valuable insight into other areas of our research, most notably new therapeutic approaches to prevent and control such inflammation. In particular, knowledge of the cellular composition and tissue environment of the retina will also allow us to design and test new approaches to suppress the action of certain cell populations, and promote recovery and restoration of normal tissue 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)?	The societal burden of AMD, Diabetes and uveitis remains significant accounting together for over 75% of blind registrations in the UK and industrialised countries and financially in the UK accounts for and estimated £2 billion/annum of health care and social welfare spend. The clinical need for these blinding ocular diseases is the requirement for targeted	

	approaches that offer the potential to modulate the immune-mediated pathology, and ultimately restore tissue function in the eye. Many current treatment modalities are limited both in terms of drug efficacy and tolerance, and therefore require alternative approaches. Through an increased understanding of the mechanisms responsible for disease progression and resolution will provide important information and guide the development of novel targets for therapeutic intervention. Equally, this work will enable opportunities for development of biomarkers to detect disease susceptibility as well as disease progression. Finally, it will also afford opportunities to provide an understanding of preventable measures that may be tested. Overall this work is applied basic science research which will deliver a greater understanding of prioritised diseases as highlighted through patient groups and major funding bodies.
What species and approximate numbers of animals do you expect to use over what period of time?	All the proposed in vivo models are induced in mice. The expected number of mice: 1000/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?	In general mice with ocular inflammation do not appear to experience systemic distress. They may experience transient or longer term loss of vision, but this does not impact on their ability to feed, groom or interact with other animals. Clinical examination commonly produces mild distress secondary to restraint or the induction of general anaesthesia. This is minimised by trained handling and by acclimatising animals to handling before commencing regulated procedures. Investigating the response of the immune system to challenge in the ocular disease models requires that a range of antigens, adjuvants, antibodies, cells and pharmacological substances are administered via systemic and local (intraocular) routes. Doses of antibodies and other immuno-therapeutics or pharmaceutical agents to be administered will be defined from all relevant information available including toxicological and any previous or established dose data determined in other models or systems. Transfer of cells or cellular fractions will be performed by intravenous, subcutaneous or

	intraperitoneal injection of 10^3-10^8 activated, antigen specific or normal control leucocytes cultured from normal or immunised animals into normal or genetically altered rodents. In all studies, some animals will receive control agents for the active reagent e.g. saline or non-active protein or carrier. On occasion (<5% of total animal numbers), it may be necessary to use irradiation and reconstitute the bone marrow recipient mice. This will enable us to construct models in which cells with targeted mutations can be analysed in the context of the whole immune response. Mice undergoing such optimised protocols, experience mild discomfort during the irradiation process, and failure of engraftment leading to systemic debility is rare (<1%). The inclusion of irradiation and reconstitution, as an optional step on the experimental protocols, raises the mild severity associated with the ocular models to a moderate severity banding. All animals will be killed at disease stages by recognised methods, and tissues collected for in vitro assessment (immune cell infiltrate, tissue structure and health).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Understanding ocular inflammatory conditions such as AMD, DR, Uveitis and retinal degeneration, and the ability to develop novel therapeutic strategies to combat blindness is severely hampered in man because of the poor accessibility of ocular tissue and fluids throughout the course of disease, and the inability to document immune responses in tissues throughout progressive disease. It is not ethical in man to obtain more than single anterior chamber sampling for research only, and not possible at all to obtain vitreous or retinal samples from patients unless the patient is undergoing an intraocular procedure that would permit sampling at the same time (which is not a common event). This is because of the relative high risk of adverse events and therefore deemed unacceptable risk to vision. As a result animals remain important for both *in vitro* and *in vivo* testing, and combined with information from any relevant human studies and genetic studies will highlight mechanisms that can be reverse

	translated to assess in man. Immune and tissue responses involve the integrated function of a number of different cell types. Many of these responses cannot be investigated in cell lines because these do not function normally, especially in terms of their growth control, a key parameter in the immune response. It is therefore necessary to obtain cells from normal or genetically altered animals for many in vitro experiments. Because the development of autoimmune and autoinflammatory diseases, and associated angiogenic responses depend on a combination of several genetic and environmental factors, a full understanding of the mechanisms that play a role in the immune responses can only be achieved by including in this research programme, studies on animals, before moving to translation of testing treatment in patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To reduce animal use, an on-going emphasis of our research is directed at developing *in vitro* assays to elucidate *in vivo* observations and test hypotheses. In the lab we have established *in vitro* platforms including Retinal Pigment Epithelium (RPE), endothelial cell, macrophage cell lines which facilitate initial investigation into how cellular phenotype and signaling pathways are modulated by potential reagents prior to testing in animals. Similarly, bone marrow from normal mice permits *in vitro* culture and polarization of macrophage populations enabling us to interrogate specific inflammatory and angiogenic responses in primary cells. In addition, we are now utilizing 3D constructs derived from primary mouse tissues (e.g. choroidal explant and aortic ring assays) which enable us to assess the potential to modulate angiogenic responses using agents *in vitro*. Many of the protocols described are common to our stated objectives, and inter-relate allowing us to perform multiple analyses from single experiments, thus reducing animal numbers further. Genetically Altered (GA) animals will be used to define pathways and mechanisms more exactly, which allows the programme of work to progress rapidly whilst minimising animal usage as the questions can be asked more directly to a particular model. For example, the using a reporter mouse, in which the resident retinal microglia are fluorescently tagged, enables us to observe and quantitate their behaviour

	in vivo, but also facilitates isolation of these cells from tissues for downstream phenotyping (genomic and proteomic) analyses. The use of GA gene-deficient mice and cells in disease models can reveal significant effect with a smaller number of animals than an equivalent experiment using, for example, blocking antibodies or pharmacological reagents.
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 design protocols that are wholly to minimise the pain and suffering, distress of lasting harm to animals. Whilst the majority of our protocols are classified as moderate, we acknowledge the welfare of animals and their wellbeing by ensuring: Animals are housed to the highest standard (temperature & humidity), within recently refurbished animal facilities (ASU) at the University of Bristol. The animals are monitored daily by facility staff who ensure a constant supply of food, water and clean bedding is maintained. In addition, daily monitoring and the close interaction between ASU staff and researchers highlights any signs of deviation from normal patterns of behaviour and/or potential distress to animals. Training of researchers is closely monitored and recorded, to ensure that all staff performing experimental procedures on animals are competent in animal husbandry and handling to minimise distress, but can also recognise potential adverse effects of an intervention and take appropriate action. This includes the use of appropriate anaesthesia and analgesia as stated in the experimental protocols. The rodent models included in this application are widely used for the study of ocular inflammatory disease and contribution of tissue damage and neovascular responses that parallel human disease for uveitis, AMD and DR. We follow established protocols that have been developed to minimise harm and maximise welfare and wellbeing of the animals. In addition to the close clinico-pathological correlate with man that these models bring, the immune system of rodents is comparable to higher animals, thus making conclusions drawn from animal studies relevant for human translation. We therefore acknowledge the need to undertake animal experimentation for purpose of improving understanding and treatments for disease in man. In doing so, we work constantly to enhance animal welfare and minimise suffering by refining models, in

	particular with respect to monitoring of disease non-invasively. This in turn minimises animal use, by improving the efficiency of the model without incurring any additional detriment to their welfare. Refinement has been enhanced by ability to use non-invasive technology. This both reduces stress to animals but also reduces number of animals required for experimentation. For example, the eye has a readily accessible window (the cornea) at the front which enables clear observation of the retina and choroidal tissues. In the past few years, enhanced, non-invasive imaging platforms have improved our ability to visualize the integrity of the tissue and vasculature, retinal infiltrate, as well as track pathological changes (tissue damage and neovascular changes), allows us to collect tissues from the mice at appropriate time-points thus ensuring maximum information can be generated from ex-vivo analyses. Importantly, these imaging techniques also enable us to accurately judge the optimal therapeutic window for disease intervention. Refined imaging platforms established in our lab include, Topical Endoscopic Fundal Imaging (TEFI) which comprises a digital SLR camera coupled to light source & endoscope provides a rapid, non-invasive screening tool that enables capture of high quality images of the whole retina without distress to the mouse or the requirement for anaesthesia, and can be performed daily. In addition, the recent acquisition of the Micron IV Retinal Imaging Microscope for rodents, delivers higher resolution and multi-parameter imaging capabilities (including Fundal imaging, Fundus Fluorescence Angiography (FFA) & Optical Coherence Tomography (OCT) modalities). Whilst FFA permits in vivo assessment of vascular integrity and detection of neovascular membranes, *t*he fluorescence modality of the system is also sensitive enough to track cells in genetic modified mice using fluorescent reporter tags, and/or visualize cells in mice that have been transfected in vivo*.* The OCT imaging permits in vivo histology, and acquisition of highly detailed cross section images of retina showing structural changes which reduces the requirement to sacrifice mice for histology during the disease course.

Project 6	**Drug and vaccine formulation and delivery**	
Key Words (max. 5 words)	Drug delivery, vaccines, formulation	
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)	The aim of this project is to design, develop and improve the delivery of drugs and vaccines within the body so to enhance their required action.	
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)?	By understanding the attributes of an effective drug or vaccine delivery system we will be able to support the development of more effective systems and translate research in this area to the market more effectively.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice/Rats. 2250/200	
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?	Mild: stress due to administration of the dose and blood sampling. The following will be taken as indicators: Reduced weight gain (10% weight loss), Reduced fluid and/or food intake, Partial pilorection, Subdued but responsive behaviour, a short term hunched posture, transient vocalisation and tremors. Schedule 1 termination.	

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are developing in vitro tools based on in vivo data we have already generated. However to study the biodistribution of these delivery systems we have no in vitro replacement. We have adopted new statistical tools (Multi-variant analysis) to replace a number of studies as we can collate and compare a range of parameters within one study.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing experiments we will initially start with small groups (pilot groups; n of 3 to a maximum of 5). We will gather the maximum amount of data from each animal and run multiple studies concurrently to reduce the numbers of controls and base line results from mice prior to dosing will also be used as controls. Based on our results and statistical analysis we will then calculate the minimum numbers of animals required such that useful data is collected.
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 vaccine studies, mice are commonly used and we will use the same bred of mouse as previous studies to ensure direct comparisons can be drawn. We will draw on previous experience and the latest research to ensure doses of antigens and adjuvants are correct and we will set the earliest possible end point for the experiments. All protocols will be performed under the mild severity limit, and animals will be monitored to ensure they do not go beyond this point. Based on previous publications, we will not use Freud's complete adjuvant as a vaccine adjuvant control due to its potential for causing distress to animals.

<table>
<tr><td>Project 7</td><td>Novel vaccine platforms targeting chronic disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Vaccination, chronic disease, VLPs, immunotherapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td><s>No</s></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The challenge of tackling many difficult targets (infectious and non-infectious diseases) has often been hampered by an inability to induce immune response of sufficient potency. The need to develop improved vaccines is important to meet these challenges. Recently virus-like particles (VLPs) have garnered great scientific interest for their ability to generate excellent responses and subsequent protection. To this end we intend to further explore the use of VLPs as vehicles for improved antigen presentation and greater antibody mediated protection. In addition we hope to investigate cost effective vaccination approaches to chronic and non-communicable diseases as an alternative to current high cost treatments.</td></tr>
<tr><td>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)?</td><td>The ability to mount an appropriate response is paramount in inducing protective immunity against infectious diseases and also desirable in tackling non-communicable diseases. As such the VLP-vaccine technology proposed has the potential to create novel vaccines for diverse conditions including Alzheimer’s, Parkinson’s, Diabetes, Psoriasis, Arthritis, Stroke, Hypertension, to name a few examples. In addition, to further develop the ability to avoid inappropriate responses when targeting self targets and to appreciate the biology involved. Lastly, simpler production and enhanced efficacy will greatly lower the costs of VLP-based therapeutics in comparison to current biological drugs thereby increasing affordability for their</td></tr>
</table>

	adopted use by the healthcare communities.
What species and approximate numbers of animals do you expect to use over what period of time?	Majority of the studies will involve vaccination and short term investigation of antibody generation; and are predicted to use a total of 3000 mice over the duration of the licence (5 years). Studies typically last no longer than 3 months in the animals – although this is dependent on disease model being investigated, up to maximum of 18 months as will be required for example, in studies relating to Parkinson's disease.
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?	VLPs have been in use for several decades and have demonstrated excellent safety profiles. Therefore following vaccination with VLPs we expect minimal adverse effects to be observed, limited to local irritation or swelling typical of any needle stick injury. Discomfort is therefore short-lived and only mild swelling at the site of injection which quickly resolves. The immune response will be monitored periodically by sampling the blood and measuring antibody production (seroconversion), and at the end of the study the animals will be humanely killed. To maximise the amount of information generated from each animal, further tissues samples may be collected to investigate cellular responses and further elucidate the mechanisms involved.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Despite great advances in understanding, immune responses remain a highly complex cascade of events that as yet cannot be accurately replicated outside the living organism. As the development of vaccines are destined for use in animals and humans, we need to test their safety and efficacy in a living system before they can progress towards clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Robust seroconversion as an output will allow us to use the minimum number of animals per group Logical design processes will be employed to minimise vaccine candidates and avoid pointless waste of animals Wherever possible we will seek to ensure maximum information is gathered per animal per experiment to inform future studies and limit group numbers

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 models are currently amongst the best for investigating the immune response and their relationship with human system are well characterised and documented. Several animal models are available to investigate particular branches of the immune response. This background experimental knowledge allows for shorter protocols to be designed and refinement to be practiced. Wherever possible we will seek to employ administration routes of least pain and potential for further complication. Pain relief and careful observation following invasive procedures will be applied as required following expert advice from skilled and experienced veterinary staff.

Project 8	**Immune responses to persistent herpesviral infection**	
Key Words (max. 5 words)	Memory inflation, cytomegalovirus, adenoviruses, vaccine	
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)	Use of persistent viruses to stimulate specific immune responses that might provide long-lived protection against infectious challenges.	
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)?	Overall, we aim to fully define the function and in vivo protective capacity of responses initiated and sustained by recombinant herpesvirus vectors and the adenoviral model. This could be of major influence in the development of new vaccines for a variety of diseases. It additionally adds to our understanding into the field of immune ageing.	
What species and approximate numbers of animals do you expect to use over what period of time?	We consider this to run over a 5-year period. All experiments will be in mice, and total experiments will look to use 6000-8000 mice including all breeding procedures.	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice will typically show minimal signs in response to the vaccination, but some may display short-lived signs of systemic illness including piloerection, altered movement, diarrhoea and body weight loss after administration of infectious agent. The frequency of this adverse event depends on the infectious agent, the strain and immune status of the mouse. These adverse effects are most likely to occur in those mice which are challenged with infectious agents where they have not been	

	previously immunized with a protective vaccine, We expect all animals to experience no greater than a moderate severity level, and in reality we expected the greater proportion of animals to only experience mild severity procedures. At the end of the study, the animals with be euthanized using a Home Office approved (Schedule 1) method or exsanguinated under terminal anaesthesia We will remove the relevant organs (liver, spleen, lung) to assess the quality of the immune response throughout the animal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The question being asked is a physiological one, which requires analysis of an immune response in vivo. It is not possible to address the importance of specific factors in vitro, even though we will maximise the use of in vitro assays to confirm key findings, and continue to do studies in detail of human immunity including vaccine challenges. The study of human vaccine recipients is an important component of the overall study, with the aim of translating the findings observed in the mouse, into new adenovirus or cytomegalovirus based vaccines in man.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Over time we have a developed knowledge of this work, this being a licence renewal. We have previously established the minimum required number of mice to assess a scientifically meaningful result and intend to continue to work upon this framework. The group have worked with biostatisticians to evaluate the use of new techniques based on analysis of the whole mouse genome, which can be applied to experiments to provide the maximum amount of information from small numbers of animals (minimum of 3 per group). Only the minimum required amount of animals will be used in any one experiment and efforts will always be made for those members of staff working within this project to where possible share or combine usage of mice.
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	The mouse is a very well defined model for immunologic experiments and the model proposed using MCMV is highly reproducible and therefore can be used more efficiently and with the maximum refinement and reduction. The use of existing genetic models to probe the mechanisms of antigen presentation and T cell activation / homing make the

minimise welfare costs (harms) to the animals.	mouse model especially appropriate. None of the protocols involved in this work are classed as severe. In particular we will focus on the use of the adenoviral vector model, which has the most limited impact on the animal's welfare and provides clinically relevant data. To minimise welfare costs we will work closely with the animal care team to provide the best environment for the mice, and ensure appropriate monitoring is in place to continuously assess their health status. This monitoring will be tailored for the experiment and will be reviewed regularly to ensure it is suitable and allows the team to limit any harm to the animals.

Project 9	**The immune response in wound healing and cancer**	
Key Words (max. 5 words)	Inflammation; wound healing; cancer; mice; zebrafish	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In general terms our aim is to learn more about the inflammatory cell response to tissue damage and/or presence of transformed cells, since there is substantial evidence that this immune response has a profound influence on the outcome of both the healing process and of cancer progression. We want to know precisely how immune cells interact with stromal cells at wounds and in a cancer setting in order to uncover “targets” for therapeutic intervention that might prevent scarring or enhance healing of chronic wounds, and block inflammation mediated cancer progression.	
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)?	We envisage that our programme of work for the next 5 years will reveal more about the mechanisms underpinning normal healing of skin and other tissues and how this goes wrong when healing fails eg as in a chronic, non-healing wound, or in scenarios where major scarring results. We hope to uncover what are the key genes and signalling pathways, particularly those associated with the wound inflammatory response, which could be modulated in order to improve healing quality in the clinic. Similarly, we aim to discover how one might modulate the cancer inflammatory response in ways that lead to cancer death rather than progression, and ways to modulate the inflammatory response after the standard cancer treatments of surgery and radiotherapy to enhance cancer killing.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mice – 1700 Rats - 100 Zebrafish – 5400 (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?	Our standard murine neonatal and adult skin lesions are generally re-epithelialised in approximately 7 days and appear to cause little if any discomfort. For those where we inoculate with bacteria we expect some delay in healing but no systemic bacterimia. For all our wound studies, animals will be carefully monitored for deviations from normal behaviour that might indicate pain or suffering, or systemic infection, and our NVS consulted if this were the case. Appropriate control measures are in place to ensure that all animals will be monitored closely and appropriate action taken. All animals will be killed at the end, either by terminal anaesthesia or an approved humane method. We consider these wounding studies will never extend beyond Moderate severity. Our murine embryo wounding studies are performed at a stage prior to functional sensory innervation and so will perceive no pain. And the blood collection from rats that we need for serum to culture the mouse embryos will be performed under terminal anaesthesia. Our highest severity adult zebrafish studies will involve lesioning of the skin or heart and subsequent live imaging under the microscope whilst still under anaesthetic. In general we have seen no adverse effects in operated fish and they recover and feed soon after coming around from anaesthesia. All animals will subsequently be killed by terminal anaesthesia or an approved humane method. We will breed some fish in ways that lead to their developing tumours and these fish will be carefully monitored and killed before the tumours adversely effect their behaviour including feeding. For some of these animals we will surgically remove part of the tumour or treat with radiotherapy (whilst under anaesthesia), and if these fish showed signs of distress or unusual behaviour post these treatments they too would be immediately killed. Again, we consider these cancer studies will never extend beyond Moderate severity.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever we can we use in vitro models and our lab has also pioneered the use of Drosophila as a model of wound inflammation. However, in vitro studies can never completely replicate the complex interactions that occur in vivo and many aspects of wound and cancer inflammation can't be fully modelled in flies; for example, Drosophila have only one "leukocyte-like" lineage, the hemocyte, and no blood vessels (so can never model the rate limiting diapedesis step in which leukocytes are drawn from the blood stream) and have no true chemokines (immune cell attractants) in their genome.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all of our wound healing and cancer studies we use the minimum number of animals possible to provide rigorous, statistically significant datapoints, based on previous work and power analysis. We consult regularly with University of Bristol statisticians about appropriate N's for our studies and we consult with colleagues outside of Bristol doing similar experiments. Results will be monitored as experiments are undertaken to determine whether subsequent experiments could use fewer animals if possible.
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 also use Drosophila as a wound inflammation model but due to some fundamental differences in their immune system (eg, only one "catch all" lineage of immune cell - rather than several lineages of innate and adaptive immune cells in vertebrates - and no vessels and so diapedesis of immune cells through vessels does not occur), we must use vertebrate models to investigate some of the complexities of the inflammatory response to tissue repair and cancer. Our move into using zebrafish has meant that we can reduce the numbers of mice that we now need for our studies (reflected in our request for fewer than half the numbers of mice/rats than we requested in previous PPL application), but, again, not all the complexities of mammalian skin healing and inflammation will be perfectly modelled in a fish and so we need to continue some murine studies. Therefore, we feel that our use of different model organisms allows us to reduce and refine the number of protected animals we use as much as possible. The majority of the animals we require are only used for creating and maintaining lines where specific cells are labelled with a fluorescent marker. In comparison,

	a relatively small number are required for wound healing experiments where we can study the role of specific immune cells in the healing process. To minimise harm to the animals, they are monitored daily (more often when undergoing procedures) and where there is any concern, advice is immediately sought from the Named Veterinary Surgeon and Named Animal Care and Welfare Officer, before appropriate action taken.

Project 10	**The Causes and Treatment of Autoimmune Diabetes**	
Key Words (max. 5 words)	Diabetes, insulin, immune system	
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)	Type 1 (autoimmune) diabetes occurs when the immune system attacks and destroys the insulin-producing β cells in the pancreas. Currently, patients are treated with insulin but this is not a cure, and in some people, maintaining good metabolic control is extremely difficult. In the long term, people with type 1 diabetes can develop kidney failure, blindness and die of early heart disease. Therefore, it is important that treatment is developed to prevent this devastating disease. The aim of this project is to define the role played by the CD8 T(killer) subset of white blood cells that recognise insulin and attack these insulin-producing β cells in the pancreas. This information is important for future development of immunotherapy to prevent diabetes. Cells recognising insulin can be found in the early stages of diabetes in the pancreas. In our programme, we aim to use understanding of basic causes and how the diabetes develops in order to inform best design new therapies that specifically target damaging immune cells: 1.Define how the insulin specific killer CD8 T cells are regulated when insulin expression is altered genetically and how the cells become activated to attack the pancreas. 2. Define how B regulatory cells which can reduce activity of damaging killer cells develop and how to enhance their function. 3.Define the effect of modifying killer cells so that they can specifically target and kill or regulate groups	

	of T cells that recognise insulin and other self antigens and to test if this will delay or prevent diabetes. 4. Define the best ways of delivering therapy that is directly translatable to humans to stop the cells that can damage the pancreas causing diabetes.
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)?	Benefits from this project include better understanding of the development of type 1 diabetes as the model that we use has some very similar features to the human disease. We will also test whether we can prevent and cure diabetes using a number of different strategies. If these are successful, the aim is to take this in the next steps towards applying these strategies to human diabetes.
What species and approximate numbers of animals do you expect to use over what period of time?	We have several individual parts to the project and expect to use expect to use 30,000 mice over the next 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?	Most of the animals will not experience any adverse effects. Some animals may experience minor transient discomfort when an injection is given. Some animals will develop diabetes, where they will become thirsty and pass more urine. They may start to lose weight. We will either treat them with insulin to control the diabetes, or they will be killed at this point, before they have significant illness or weight loss. The expected level of severity is “mild”. A small number of animals will undergo surgery for transplantation, for which they will undergo an anaesthetic and have pain relief on recovery. The level of severity is “moderate”. At the end of the experiments, the animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will study aspects of development, where possible, in vitro. We cannot investigate whole immune system and the paths that cells take in humans who have diabetes and we are unable to manipulate the conditions that change development of T cells in humans and many of the experiments in this section will be performed *in vitro*. We will use cells that arise under altered selection conditions *in vivo* to test whether the capacity to cause diabetes has been altered. This has to be done *in vivo* as diabetes arises as a complex procedure that involves numerous cell interactions and the

	metabolic state that cannot be recreated *in vitro*. Diabetes occurs because cells migrate to the pancreas, enter the pancreas and attack the insulin-producing cells. The influences within the whole mouse that affect all these processes cannot be studied in tissue culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have made every effort to reduce the number of mice that will be used for these experiments by using protocols that require a lower number of animals for each experiment. We have recently developed a protocol that reduces the number of donors required for experiments by optimizing cell expansion in vitro. We use the minimum number of mice that will give us sufficient information based on statistical considerations. We have the advice of a statistician in planning as well as at the stage of reporting our results.
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 species of choice here because of the availability of strains and the immunological reagents that allow investigation of development of immune T and B cells, together with the ability to track regulatory cells. They are also important in the study of responses that are similar to human immune responses. Many of the mice used do not have any obvious abnormality that affects their day to day existence. Some mice are known to develop diabetes and for the most part, apart from mice that will be treated with insulin, they will be studied before development of disease or within a short period after diagnosis. There are mice in which the immune system is deficient which are necessary to allow us to study the cells recognising insulin and other self antigens without interference from other immune cells. All mice will be housed in isolators, individually ventilated racks or filter framed cages to reduce the risk from infection.

<table>
<tr><td>Project 11</td><td>Intrinsic & extrinsic effects on B cell responses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>B cells, vaccination, mice, antibody</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Maturation of B cells able to produce high affinity antibodies specific for pathogens is the central mechanism by which vaccination protects us from infection. High affinity antibody is produced by long lived plasma cells and these are replenished upon reinfection from long term surviving memory B cells. We try to understand
- how high affinity B cells form and are sustained to become long live memory B cells and plasma cells.
- which signals regulate this.
- how these processes are affected by ageing.</td></tr>
<tr><td>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)?</td><td>This project is basic immunological research and so will not produce any immediate benefit to patients. Understanding these basic processes may lead to new ways of manipulating antibody responses: to produce more efficient vaccines, produce drugs that may improve the antibody response during infection or sustain longer lived antibody production and to find ways of reversing the reduction of antibody responses after infection and vaccination in the aged immune system. E.g. for influenza it may lead to cross-protective vaccines that work over several annual epidemics with newly emerging virus strains, or protect the elderly over sustained periods.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse.
Breeding 16,000. Experimental 6700 over 5 years</td></tr>
</table>

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?	Because our work is on the immune system the genetically altered (GA) mice that we breed and study on this licence have changes that may produce an increased susceptibility to infection. However, in order to study the immune response our mice are kept in very clean environments and this means that infections are very rare. When mice are handled, such as during health checks and vaccination, they will experience stress and discomfort. All our GA mice have small tissue samples for genotyping and identification taken, normally by ear clipping, which will induce minor pain. In a small number of animals genes will be induced by feeding the drug Tamoxifen – an estrogen analogue that can lead to weight loss. To study immune responses we vaccinate mice, which, as in humans, produces pain from the injection. To see how immune responses progress blood samples may be taken repeatedly which can be uncomfortable mainly due to handling and restraint. A small number of mice will be irradiated to wipe out the immune system. This will immediately be followed by transfer of immune cells from healthy normal or genetically altered mice . Irradiation also affects other body systems, such as the gut, and mice will be monitored to avoid weight loss. To produce stem cells from which B cells originate a small number of animals will be treated with the chemical 5-FU. Side effects from this are not expected within the time scale of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The differentiation of B cells happens in incredibly complex tissues consisting of many different cell types, some of which are still completely uncharacterized. It also involves migration of B cells between different microenvironments with complex rules on cellular interactions in certain spaces and at specific times. There is currently no way to remake these environments *in vitro*. There are currently better attempts available to simulate these environments *in silico*, and we are actively involved in developing these *in silico* models. However, this again involves *in vivo* experiments to predict rules for *in silico* modelling and then *in vivo* experiments to verify the *in silico* data. As we study the behaviour of immune cells in their tissue environment, and the tissue environment provides a

	plethora of signals to the immune cells, it is impossible to do this work in vitro and produce anything meaningful.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To maintain our mouse colonies but keep the numbers of mice low breeding of animals is reduced to the absolute minimum numbers of breeding couples necessary to safely maintain a colony. This is usually 2-3 breeding pairs, with larger numbers being produced when experiments are being prepared. Experiments will involve minimum group sizes to produce statistically significant results. This is the minimal number of mice necessary to obtain significant results, as predicted from power calculations. Because we study how B cell responses evolve with time, frequently time course experiments will be performed. For time course experiments only time points when key cellular differentiation events happen will be studied.
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 our studies we use mice. As mice are mammals they have an immune system that is very similar to humans. Non mammals and invertebrates are not useful for our studies as they either do not have B cells, or their immune system is less related to the human system. Of all small mammals mice are the species of choice because they have the best studied immune system, and because of the large number of tools available to study and manipulate their immune system. There are many experimental mouse specific reagents, e.g. antibodies, and genetically altered mice.

Project 12	**Biology of carbohydrate-binding proteins**	
Key Words (max. 5 words)	Immunology, cell biology, glycobiology	
Expected duration of the project (yrs)	Five	
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)	Carbohydrate-binding proteins are expressed on cells of the immune system to fight against infection and cancer. Also they are involved in autoimmune diseases such as multiple sclerosis and arthritis. The objectives of the proposed project are as follows. 1) To understand roles of carbohydrate-binding receptors in the immune system. 2) Assess the effect of such carbohydrate-binding receptors on human diseases such as cancer, infection, and autoimmune 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)?	Accomplishment of objective 1 will provide a deeper understanding of how our immune system responds to bacteria, virus, and other stimuli. Such information may provide a basis to develop novel treatments of human diseases such as cancer and infections. Accomplishment of objective 2 will enhance development of a novel treatment for human diseases. For instance, compounds capable of strengthening our immune function discovered in this research may be useful to treat infectious diseases and cancer.	
What species and approximate numbers of	Mice, estimated number of animals 7160	

animals do you expect to use over what period of time?	Rat, estimated number of animals 30
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 experiments in the proposed project will impact *general wellbeing* of animals at moderate level. Invasive procedures include injection of substances into mice, some of which may cause discomfort and illness e.g. abdominal pain or diarrhoea, immunization of substance into rats to generate antibody of interest, injection of toxins into mice that cause symptoms of illness but have been weakened so that they don't kill animals, and non-recovery surgery in mice under anaesthesia, from which tissues will be used for experiments. Small number of mice will be expected to show a sign of illness in response to induction of diarrhoea and bacterial infection. Such experiment will be performed only when it is imperative to achieve the objectives. In such experiment, animals will be closely monitored and early humane end points applied such that the animals will be promptly killed to avoid unnecessary suffering. At the end of experiment all animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Considering most of the data we look for in this project rely on the host's own immune system, experiments using animals are particularly required As the complexity of interactions of different body systems cannot be reproduced in a laboratory cell culture setting. It is imperative to obtain information regarding to where in the body and when carbohydrate-binding proteins play roles in the immune system. Such information will be only achieved by experiment using animals. As alternatives, experiments with human biopsy and cell lines will be employed wherever applicable and before animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before starting regulated procedures, statistical analysis will be performed to determine the minimum number of animals used in the experiment. The experiment will be designed in a way to collect as much as information with minimum number of animals used. Discussion with other researchers and extensive literature search will be conducted to reduce the number of animals being 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 (harms) to the animals.	Mice are the most extensively studied species for immunology research with relevance to the human system well established, and many genetically modified strains are already available. Importantly most of the carbohydrate-binding proteins we study are conserved in human and mice, rationalizing the use of mice as a model system. We also use rats to generate monoclonal antibodies required to achieve the objectives. Considering the need for antibodies against both human and mouse, rats are appropriate choice. In order to minimise animal suffering, animals will be closely monitored and any animal displaying any signs of suffering will be humanely killed. We will refine procedures as much as possible based on updated best practice.

<table>
<tr><td>Project 13</td><td>Parameters of macrophage origin and function</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Macrophage, ontogeny, stem cell, inflammation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Macrophages are immune cells that reside within body tissues. They have key roles in maintaining healthy tissues but also contribute to most of the damaging processes that occur during disease, raising the question of how they manage to perform such diverse functions.

While some macrophages come from the blood during inflammation, those that reside in healthy tissues proliferate rather than recruit blood cells into their ranks. Even during disease it appears resident cells self-regenerate in order to maintain a distinct population from the recruited blood cells. The aim of this project is to establish the mechanisms through which resident macrophages are maintained in the tissues and to determine if separate types of macrophages perform unique and non-overlapping functions.</td></tr>
<tr><td>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)?</td><td>This project will lead to a greater understanding of how macrophages perform diverse functions during health and disease and if/how we might control certain functions by targeting individual populations. Identification of a stem-like cell for tissue macrophages would provide a target to expand or contract the resident macrophage pool during disease.</td></tr>
</table>

	Ultimately, information from this project will contribute to strategies to block damaging properties of macrophages during inflammatory disease while retaining or enhancing their beneficial functions.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 12050 mice over the 5yr period 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?	Many of the animals will be used in breeding programmes for GA mice. For experimental animals: We will use several diverse inflammatory disease models that effect either the body cavities, lung or liver to determine common features of resident macrophage biology across different tissues and inflammations. While all models are classified as moderate, most are well tolerated.. Animals may experience effects of moderate severity and will be humanely culled before this limit is exceeded. Chronic liver injury results in progressive liver pathology and may lead to signs of moderate severity such as hunched posture, piloerection and reduced mobility,, although within the timeframes detailed in this proposal no animals should experience liver failure. Animals will be visually monitored and weighed, and any animal beginning to show signs of weight loss will undergo heightened surveillance. Animals will be humanely culled before they exceed the moderate severity limit. Injection of cells and reagents will cause minor discomfort but with low risk (< 1%) of additional complications. Substances will be administered at non-toxic doses, based on experience and or published literature, and at volumes in accordance with best practice. Whole body irradiation can result in moderate symptoms of radiation sickness, such as transient diarrhoea and loss of immune function. Short term treatment with antibiotics, special diet, and housing in a barrier environment will prevent/counter such effects. Failure of donor BM to engraft is extremely rare but results in mice becoming permanently immune-compromised. Such animal will be identified and humanely culled by daily monitoring for signs of deterioration in the first few weeks post-

	irradiation. For surgical procedures to implant cells or donor tissue that have been depleted of cells, or other procedures involving anaesthesia, complications will be avoided by correct dosing of anaesthesia and using heat pads or insulating coats during and after the procedure to maintain body temperature. Following surgery, pain will be controlled by analgesics. Good sterile technique will be used to circumvent the risk of infection. Implantation of human or mouse tissues that have been depleted of all cells is not expected to cause additional adverse effects to those that rarely result from minor surgery. Immune cell depletion may increase risk of infection. If required animals will be housed in barrier environments and may be treated prophylactically with antibiotics. Not more than 10% of total blood volume will be withdrawn at any occasion and no more than 15% of total blood volume in any 28-day period in order to avoid hypovolaemia or anaemia. Any mice exhibiting unexpected adverse effects or clinical signs likely to exceed moderate severity (e.g. severe weight loss or combinations of other visual signs such as hunched posture, inactivity, raised fur) will be humanely sacrificed. If in doubt, the advice of the veterinary surgeons will be taken. At the end of all procedures animals will be killed humanely by a schedule 1 method or under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Immune responses are a complex series of interactions between multiple cells types that occur sequentially and simultaneously within several different tissues. To understand such complexity is it essential to analyse responses in live animals since this cannot be studied in a meaningful way in a plastic dish, particularly when trying to identify the origins of cells present during inflammation. The types of analysis we require e.g. where a cell has come from, how many times it has proliferated and what it's function is during inflammation, is not possible by simple analysis of human tissue samples since biopsies often represent only a

	single snapshot in time and provide little in the way of historical information of a cell. Attention will be given to developing methods that may provide this information, for example by assessing host *vs* donor origins of cells in organs that have been transplanted into human transplant patients, but without increasing the likelihood of rejection of such organs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All biological measurements have some variability across a population of animals. This degree of variability determines how many animals are required to detect a significant difference of a particular magnitude between treatment groups. Analysis of data from previous or pilot studies will gauge this variation and determine the necessary number of mice needed to prove/disprove our hypothesis. This will ensure each experiment generates meaningful data and that we are not using too many or too few animals. In-bred mice will be used to reduce variability and thus group size. More complicated statistical analysis on data from multiple experiments will reveal subtle differences without having to increase the number of animals in each group. Sharing of tissue/data from each experiment will be facilitated by regular communication within the group on planned 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.	The mouse immune system bares remarkable similarity to ours. In particular, circulating blood macrophages in human and mouse are highly alike and tissue resident macrophages in human skin grafts been noted to stay of donor origin for many years, similar to observations in mice. Among experimental species, the mouse immune system is the best characterised with well-established markers for defined immune cell subsets and activation stages, and the availability of a wide range of transgenic mice and commercial reagents, making this species the most powerful tool to address my questions. Each model of inflammation we will use has been selected based upon its known/predicted effects to cause either proliferation or replacement of resident macrophages in the liver, lung or serous cavities, together with its ability to closely model acute or chronic pathologies in humans. These models are within the mildest methods used to study acute and

	chronic inflammation, and the response induced is reversible meaning that cessation of delivery of the inflammatory agent leads to resolution of almost all the pathology. These models have been very well defined and progressively refined over many years meaning the likelihood and types of adverse effects are minimal but known, so that animals can be appropriately monitored and appropriate precautions taken to minimise their harm. Precautions will also be taken where animals are likely to become transiently/chronically immuno-compromised (certain GA strain or irradiated mice) to prevent infection e.g. using barrier facilities, sterile techniques and maintenance on antibiotics. Appropriate monitoring will be performed. Tissue irradiation-protected chimeric mice are an accurate way to trace the origins of tissues macrophages that represents a significant refinement to whole-body lethal irradiation, as the organs (the immune and digestive systems) most susceptible to radiation damage remain predominantly shielded and thus unaffected.

Project 14	**Immunology of cancer and pregnancy**	
Key Words (max. 5 words)	Immunity, pregnancy, cancer, transgenes, mice	
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 aims to address the following immunology questions by focusing on a specific lymphocyte population known as natural killer (NK) cells, which are implicated in reproduction and cancer. In the context of Reproduction: How do maternal lymphocytes regulate blood flow in the uterus during pregnancy? What are the precise molecular interactions between lymphocytes and fetal placental cells that regulate the growth of the fetus and its placenta? Does the impact of maternal lymphocytes on fetal growth go beyond life in the womb? And is this due to an effect of maternal lymphocytes on metabolism of mother and fetus? In the context of cancer: How do genetic alterations in cancer cells impact on the interplay between cancer cells and immune cells? Do cancer therapies modulate such interplay and can NK cell improve cancer therapies? And finally, can a recently described subset of NK cells, which seem to carry immunological memory, contribute to successful pregnancy or to immunity to 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)?	We should be able to decipher the molecular mechanisms of how NK cells regulate placental and fetal growth. We will gain insights into how maternal immune celLs impact on metabolism of mother and fetus, and in so doing, how the maternal immune system may help lay the foundation for healthy adulthood. These benefits are worthwhile because they will improve our fundamental understanding of some complications which occur during pregnancy such as miscarriage and fetal growth restriction. We expect to link specific gene alterations in cancer cells with NK-ceII responses and to deepen our understanding of the interplay between cancer treatments and immunity to cancer. We will devise new ways of leading NK cells to destroy cancer cells, including those that have acquired resistance or escaped other cancer therapies. Moreover, the results of this project may help to optimise use of current treatments for cancer, as well as to identify new treatments. Existing collaborations with Academics, Industry and Oncologists will facilitate the translation of our research into the clinic.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, up to 13,300 in 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?	Timed mating, cell transfer, the use of small molecule inhibitors and the analysis of immune system in mice are protocols that have been used extensively in immunological research and do not cause harm to the mice yet allow us to extract large amounts of relevant new knowledge. The majority (>95%) of the animals that receive tumour cells are not expected to show signs of adverse effects that impact materially on then general wellbeing. No more than 5% of these animals are expected to show clinical signs of a moderate severity as a result of large tumours, when higher doses of anticancer drugs are used, or when a novel anti-cancer agent is administered. Very rarely the severity of these signs may be such that the humane end points may be reached. Mice will be humanely killed if they appear hunched, immobile after touch or display other non-transient moderate clinical signs.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Prior to embarking on animal experiments we will collect as much evidence as possible from publications and by using in vitro models of immune cell activation. However, there are no cell culture models that adequately mimic the cellular conversation we want to study. Despite the technological advances in cell cultures, animal models of tumours are still the benchmark for understanding mechanisms of diseases and treatments in cancer.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We perform statistical analysis to ensure that we use the minimum number of mice per group. When we use a novel compound, the drug will be pre-screened to determine the minimum dose that is likely to be effective. At the end of an experiment, to maximise the information from a single animal, we will collect samples from multiple body sites and provide other affected tissues to appropriate 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.	The mouse immune system is similar enough to the human immune system that data we obtain is applicable to clinical research. Pre-clinical cancer models will invariably cause some pain and discomfort to the mice. The models we have chosen are ones we judge to best mimic human disease, with well-defined end points. Arguments can be made that some of these models should also be extended to non-human primates, which may better predict the efficacy of a particular treatment strategy in humans. However, from a cost-benefit analysis point of view, we judge that we can obtain scientifically rigorous data using the mouse model and that this is the model system that best bridges the fundamental research done in the lab with further pre-clinical and clinical evaluations. To minimise harmful effects we will use models that allow regulation of the activity of the gene under study using well-established agents to induce or delete the candidate gene. In cancer models we graft genetically modified cancer cells, so that the effect of the mutations are confined to the tumour itself. When we apply anti-cancer agents and the toxicity of the agent is unknown, we will use the lowest dose on a minimal number of animals, The animals will be closely monitored and humanely killed if growth of internal tumours is suspected or other signs of pain, distress, or suffering.

Project 15	**Mechanisms of T cell survival and homeostasis**	
Key Words (max. 5 words)	T lymphocyte, homeostasis, cancer, mice	
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)	T lymphocytes are cells of the immune system critical for normal immunity. They are responsible for recognising invading organisms and orchestrating the immune response to expel such pathogens. Following the expulsion of a pathogen, it is changes in the composition of T cells that allow for more rapid and stronger response by the immune system should the same pathogen be encountered in the future. The acquisition of such memory of infections is exploited in vaccine production, for example when the memory of infections is used to educate the immune system. It is known that new T cells are generated throughout life and must be maintained in a state of readiness, perhaps for many years, before they encounter the pathogen they can recognise. The mechanisms of production, maturation and survival of T cells are incompletely understood. We plan to identify the origin of key immune hormones required for long term survival of T cells. We also aim to identify those factors within cells that are specifically involved in regulating T cell survival and death in the immune system in response to immune hormones and other ‘survival’ signals that T cells encounter in the environment of the body. We have identified a signal inside T cell that is crucial for making fully functional T cells. We now plan to investigate how this signal is switched on and off, and genes that are controlled by this signal that are	

	responsible for controlling normal T cell 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 proposed work is anticipated to give fundamental scientific insights in the processes of T cell developmental maturation and survival. Such insights will have implications for understanding immune reconstitution in humans that occurs following chemo and radio-ablative therapies. The mechanisms governing T cell survival are also commonly mutated in cancers of the blood and therefore a greater understanding of these mechanisms may identify new targets for cancer therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	From past experience, we anticipate the use of ~1000 mice for experimental procedures, and ~2000 for breeding and analysis, per calendar 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?	This program of work rarely involves procedures expected to result in adverse clinical signs. Nevertheless, some of the protocols have the potential to induce clinical symptoms and discomfort. Mice will be closely monitored for development of such signs and clear endpoints are defined in order to minimise discomfort experienced by individual animals. Animals that have minimal discomfort will be treated with antibiotics or painkillers as advised by the vet but it may be necessary to humanely kill mice that do not quickly respond to treatment. Mice that develop leukaemia will be killed humanely in order to minimise discomfort, but at the same time allow isolation of tumourous cells whose study will give new insights into the signalling mechanisms governing leukaemia development. Typically, animals are killed, using a humane method, at the end of an experiment to allow full characterisation of their immune systems following the experimental manipulation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While it is possible to generate information regarding regulation of some aspects of the immune cell behaviour in cell culture it is not possible to mimic homeostatic or immune responses to infection *in vitro.* Such non-animal systems, while useful, do not fully replicate the complexity of immune interactions or disease pathogenesis and it is essential to use appropriate and robust animal models to understand these processes. Furthermore to develop therapeutic approaches with potential to alleviate human disease it is necessary to establish parameters influencing

	efficacy in an animal model. We will remain alert to any advances, which will enable the replacement of animals. For instance mathematical modelling that will underpin some of the studies on homeostatic mechanisms in maintenance of adaptive immune cells will lead to predictions that can reduce the number of animals that will be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The efficiency of animal usage is maximised in consultation with animal technicians, by careful control of breeding to meet research needs with respect to numbers, phenotypic uniformity and health. This will be greatly facilitated by a custom built mouse database in which every breeding pair and every mouse born is recorded and through which the scientist can readily monitor the numbers of mice they hold. Together these measures ensure that numbers of mice are kept as small as possible. This programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. This highly integrative approach will maximise the information obtained from the minimum resource**.**
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 achieve our goals we propose to use the mouse as the model organism to study the development and function of the immune system for several reasons. Genetic modification of mice are well-established and their blood and immune system has been intensively studied and bears extensive similarities to that of humans. There exists a vast array of reagents facilitating the study of the immune system in contrast to the situation in other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this programme to the same extent as the laboratory mouse. Careful monitoring of strain characteristics and experimental procedures will be employed. Animals exhibiting any unexpected harmful clinical signs will be killed using a humane method, or in the case of individual animals of particular scientific interest, advice will be sought from the local Home Office Inspector.

<table>
<tr><td>Project 16</td><td>Physiological regulation of innate immune responses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Myeloid cell, Inflammation, Hypoxia, Metabolism, Mice</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>White blood cells are critical for our lung defences against infections, but if they remain in the lung they cause damage in common and serious lung conditions including chronic obstructive pulmonary disease (COPD). Neutrophils are one of the first white blood cells to reach these areas, but it is their removal, in part by macrophage uptake, that is critical in preventing this damage. Their removal is dependent upon the ability of these cells to die in a programmed manner, a process regulated by oxygen availability. We now know this process is controlled by a pathway that enables cells to detect and respond to changes in oxygen tension, the HIF/hydroxylase pathway. This pathway is also tightly linked to the energy status of cells. Relatively little is known about the interaction between energy status, oxygen availability and the immune response. We propose to investigate whether lack of oxygen (hypoxia) can regulate the ability of white blood cells to generate energy, protect themselves from damaging stresses and kill bacteria. In the longer term we hope these insights will allow development of novel treatments for these diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the</td><td>Respiratory disease kills one in five people in the UK, with almost 30,000 of these deaths a consequence of chronic obstructive pulmonary disease. To date there remains very little in the way of effective treatment strategies to target some of</td></tr>
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project)?	the most common inflammatory lung diseases typified by COPD. Consequently, they remain a significant disease burden to society. If we were able to shed light on some of the basic molecular pathways regulating neutrophil persistence at sites of inflammation and identify molecules that can selectively regulate neutrophil death and clearance, whilst preserving key anti-bacterial functions, this may be of help to the future development of effective anti-inflammatory strategies so desperately needed for the effective treatment for these common and disabling chronic lung diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We study mice because their immune system and lung anatomy is similar to man. There are many resources available for use with mice and genetically modified mice are available to test key factors controlling the immune response. We test key hypotheses in human cells or in patient samples *in vitro* before studying mice to reduce numbers. We collect the maximum possible number of samples to reduce mouse usage. We refine our methods to ensure reproducibility and calculate group sizes carefully using validated statistical methods with the input of a statistician. We estimate we will use approximately 40700 mice in the lifetime of this license.
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?	Mice will most often receive bacteria directly into the lung, which requires a short surgical procedure under anaesthesia to identify and put a small cannula into the windpipe (intratracheal instillation). Mice recover rapidly and there is only minimal suffering. This model is used since it most closely mimics the aspiration of bacteria by humans. For many of our studies we use low doses of bacteria and infection is sub-clinical. When we do establish pneumonia we try and ensure that the disease is not too severe. All mice are carefully monitored for signs of ill health and are culled before they show any signs of distress. When we establish infection at a different site, to compare the response in the lung with other sites, we will also monitor closely for any signs of ill health.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We require mice because we cannot study complex multicellular interactions in cell cultures. Experiments using multicellular human tissues are not yet refined enough for our studies while

	important differences between invertebrate and vertebrate organisms mean we cannot replace mice with invertebrates, although we will continue to explore their potential use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will refine the models outlined above to reduce assay variability thus reducing group sizes. We will continue to explore the potential of small animal imaging to allow collection of data at multiple time points and will maximize and refine the collection of multiple pieces of data from individual mice. Enhancing reproducibility and collecting multiple data points from individual mice also reduces numbers.
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 investigation of the early stages of infection and inflammation ensure minimal suffering. The intratracheal instillation of bacteria involves a short surgical procedure without ill effects. Breeding mice with genetic modifications to study the working of the immune system does not involve the creation of major congenital defects or clinical illness and mice do not have features of ill health. If the mutation makes them susceptible to naturally occurring infections these are prevented by filtering air and the use of antibiotics. Similarly although bone-marrow transplantation involves conditioning with radiotherapy the treatment is broken down into low doses to minimize adverse effects and natural infections are prevented with filtered air. Modifying diet to a western style diet does not cause ill health. The wound-healing model is the least invasive way of studying tissue repair under conditions of altered inflammation and with minimal distress and rapid mouse recovery.

Project 17	**Molecular regulation of the immune response**	
Key Words (max. 5 words)	Inflammation, Cancer, Viral infection, Immunology	
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 cells of the immune system play extremely important roles in a very broad range of diseases including all immune and inflammatory diseases as well as many cancers. In these disease contexts, as well as in normal immune responses, cells of the immune system need to get the right place at the right time and this process of cellular navigation is regulated by proteins called chemokines. We have studied chemokines for many years and the purpose of this licence is to allow us to further develop these studies and to try to understand how chemokine function is regulated in health and disease and how this contributes to the establishment of immune and inflammatory 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 project)?	All our studies are of central relevance to an understanding of immune and inflammatory diseases and cancer. Many inflammatory diseases such as psoriasis and rheumatoid arthritis would benefit from novel therapeutic approaches and the insights to be provided by our studies will be invaluable in the development of new therapies for these difficult diseases. In addition we have a particular interest in studying the role of inflammatory cells, and chemokines, in cancer and believe that these molecules represent a novel therapeutic target in cancer which offers great promise. This is particularly true for metastasis which is the late stage of cancer. Metastasis is the process of cancer 'spread' which ultimately is what kills approximately 95% of cancer patients. Metastasis remains untreatable at present. We have novel insights into this process which we	

	believe will help us to develop new therapies for metastasis. Such a development will have major implications for cancer survival rates.
What species and approximate numbers of animals do you expect to use over what period of time?	Our studies exclusively use mice and we anticipate using about 20,000 mice over the 5-year timeframe 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?	At the end of all procedures under this license animals will be euthanased. 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 'substantial' 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 killed to prevent suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune and inflammatory responses are complicated involving numerous different cell types and molecules. These are carefully controlled in an intact animal in ways that cannot be modelled using non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have 30 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.
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 ideal for our studies as 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, they will be monitored regularly for routine signs of ill health or distress. Anaesthetics and analgesics 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. When animal suffering reaches a pre-determined level they will be removed from the study and euthanised to prevent further suffering.

Project 18	**Regulation of immunity, inflammation and haematopoiesis**	
Key Words (max. 5 words)	Immunity, asthma, infection, cytokines, haematopoiesis	
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 immune system has evolved to protect the host and fight disease. However, there are occasions when the weaponry available to the host immune system fails to combat the attack. For example, some bugs (pathogens) have developed highly sophisticated counter-measures that defeat the host. In other cases the immune system suffers from friendly fire, resulting in “auto-immunity” such as inflammatory bowel disease, or the firestorm may be inappropriately set off e.g. when pollen or house dust mites cause allergy and asthma. It is also important that the body activates peacekeepers and repairers to ensure that the damage (inflammation) doesn’t generate excessive collateral damage. Whilst we understand many of the factors that protect the host we are still unable to prevent or effectively treat certain immune and inflammatory diseases. This is because our knowledge of how cells of the immune and blood system communicate with each other, and with other cells of the body, is incomplete. Indeed in the last 5 years we have identified a previously unappreciated immune cell that may be a key player in immune disease and represent a new therapeutic target. Our aim is to define how such cells interact in the immune response and understand how they can go wrong. The key aim is to identify new pathways that can be targeted with drug treatments with the objective to improve human	

	health.
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)?	**1. Scientific/academic benefits** The core benefit of the work will be that we will increase our fundamental understanding of the immune system. A better understanding of these mechanisms will allow us, and the scientific community, to develop novel approaches to modulate immunity and blood formation to prevent disease. **2. Translational benefits** The important long-term goal of our research is to contribute to the development of therapies to both prevent and treat these diseases. We have already identified new therapeutic targets and developed drugs that are in preclinical development or moving into clinical trials for the treatment of asthma and allergy. However, many questions remain in our understanding of these complex immune reactions and it is important, given the attrition rate of potential drugs in development that we continue to pursue new treatment strategies. **3. Societal/health/economical care benefits** In the longer-term we expect our findings to inform and guide new therapies in a variety of human diseases including autoimmunity and obesity.
What species and approximate numbers of animals do you expect to use over what period of time?	All our *in vivo* experiments are performed in mice and many of our other experiments *in vitro/ex vivo* require cells and tissue from donor mice. We expect that the research programme will require approximately 116,900 mice over 5 years. A small number of rats may be used to produce antibodies and maintain a worm parasite that is necessary for our work (up to 100).
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?	Our experiments in mice will mimic, in an experimentally controlled fashion, the various scenarios the immune system has to deal with (vaccination, infection, autoimmune disease, asthma, inflammation). The majority of mice are expected to experience only very mild, if any signs of discomfort. However, as we need to understand how the immune system fails and how to avoid this, some animals will develop moderate signs of disease such as for example loss of up to 20% of their body weight, or swelling of digits of their feet due to arthritis. A few animals might experience severe clinical signs as we are using model systems of arthritis (swelling

	of the joints), multiple sclerosis (partial paralysis) and colitis/inflammatory bowel disease (weight loss with bloody diarrhoea). We will ensure that the experimental design will keep the number of mice that experience any form of discomfort as small as possible. At the end of the experiment the mice will be killed, and whenever appropriate the tissues will be collected and further analysed *ex vivo* to maximize the amount of data collected in each experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst we can study individual aspects of the immune response *in vitro*, our findings need to be validated using *in vivo* model systems since the more intricate mechanisms regulating immune responses can only be studied in the context of a whole animal. In particular, translational aspects of our research, such as the modulation of the immune system are only possible in an *in vivo* context. We are constantly exploring new *ex vivo* model systems to minimize the numbers of animals required for *in vivo* experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All of our work is based on extensive *in vitro* studies and literature review. By the time we perform experiments with mice, we usually have a good understanding of the biological processes involved. The types of manipulation we use *in vivo* will be guided by studies conducted on tissue cultures, thus refining our approach before animals are used. All experiments will be designed to minimize the number of experimental animals involved. All researchers involved in the studies covered by this application have attended several courses on experimental design and statistical approaches. Furthermore, a statistician is consulted whenever necessary.
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 are using a number of interventions that mimic human diseases including asthma, inflammatory bowel disease, viral infection and bacterial infection. The mouse has been chosen because it is the "lowest" mammalian species for which high quality reagents are available and which has immune and blood systems that closely resemble those of humans. This makes the data obtained in the experiments more reliable, which helps to reduce the number of animals. We will always consult existing

	literature to ensure that we use the latest refinements and aim to use the shortest duration and lowest severity models of disease that will yield satisfactory data. The work will be carried out in dedicated, state-of-the-art facilities by highly trained technicians and scientists, all of whom are dedicated to the highest standards of animal welfare. Tight control over the breeding programme minimises the numbers of surplus animals produced, whilst robust experimental design enables us to generate statistically valid results from the minimum requirement of experimental stock. The scientists and technicians work closely with Named Veterinary Surgeons to ensure that animals are exposed to minimal adverse effects.

Project 19	**Mouse antibodies for biomedical research**	
Key Words (max. 5 words)	Immunisation mouse antibody adjuvant	
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)	1. The generation of reagents for laboratory use; 2. The measurement of the immune response to a potential vaccine or other immuno-therapy	
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)?	Monoclonal antibodies are vital reagents for research. They can be used in assays to measure the amount of a specific molecule in a biological sample, and to localise that molecule within cells and tissues by microscopy. They can also be used to purify the molecule from a complex mixture, allowing its detailed characterisation and determination of its biological role. By studying the types of antibodies generated after immunisation by a potential vaccine or therapeutic agent, we can gain insights as to whether this is likely to work against its target disease and whether its components can be simplified further, thus reducing the risk of adverse reactions.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 300 mice over the course of five years.	
In the context of what you propose to do to the animals,	We will immunise mice with the target molecules, cell extracts or intact cells, with or without an “adjuvant”	

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	(a preparation intended to boost the immune response). We will take small blood samples over the immunisation period, to monitor the course of the immune reaction. The animals will be killed humanely and the spleen removed for the generation of monoclonal antibodies in the laboratory. Very few adverse effects are expected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune response to a particular challenge refines itself over the course of several weeks so that, at the end, very specific antibodies are being introduced. This selection process cannot be mimicked in non-animal systems, at least with a usable efficiency. All the “downstream” steps, of generating sufficient quantities of the antibodies for use in research, are indeed conducted without the need to use animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	From experience, 3-6 mice are sufficient to generate a good range of antibodies to a particular target.
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.	Some adjuvants can cause local inflammation at the site of immunisation. The use of these will be avoided unless it is clear that it is necessary for the generation of antibodies to the intended target molecule.

Project 20	**Finding curative therapies for blood cancers**	
Key Words (max. 5 words)	Blood, haematology, stem cells, blood cancer	
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)	Haemopoietic stem and progenitor cells (HSPCs) reside in regions within the bone marrow containing very low oxygen levels where they self-renew and produce all blood cells. These low oxygen levels, called hypoxia, activate hypoxia-inducible pathways in normal HSPCs allowing them to survive and self-renew and sustain blood production. In blood malignancies such as acute myeloid leukaemia (AML), disease-associated gene mutations force unusual self-renewal in HSPCs which generate difficult to eradicate leukaemic stem cells that cause leukaemia. Our new data suggest that mutations present in AML patients hijack hypoxia-inducible pathways to force unusual HSPC self-renewal and generate leukaemic stem cells. Supported by multidisciplinary collaborations, we will investigate the role of hypoxia-inducible pathways in leukaemic stem cell functions. The objective of our studies is to understand how normal stem cells in the bone marrow make blood and to identify factors that allow generation of leukaemic stem cells. Better understanding of stem cell biology should allow us to find better treatments for blood cancer.	

	We will address the following specific questions: 1) How do hypoxia pathways regulate normal stem cell functions? 2) Which leukaemia-.associated mutations affect hypoxia-inducible pathways? 3) Does enhanced activation of hypoxia-inducible pathways cause leukaemia and leukaemic stem cell drug resistance? 4) Does inhibition of hypoxia-inducible pathways erturb generation and maintenance of leukaemic stem cells and allow their eradication? 5) What are the molecular mechanisms through which hypoxia-inducible pathways drive leukaemia?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our work will directly enhance the knowledge about how blood stem cells and progenitor cells function in vivo. We will elucidate the molecular pathways that sustain stem cell survival and self-renewal. This work, in collaboration with clinicians, may lead to the development of protocols to expand stem cells for therapeutic transplantation, where their numbers are limiting. Furthermore, our work will directly identify novel pathways that are perturbed in normal stem cells and generate leukaemic stem cells that fuel leukaemia. By understanding functions of leukaemic stem cells better we should be able to identify novel therapeutics for blood cancers and potentially other cancers. Once we successfully perform experiments described in this licence we will be able to collaborate with haematologists to desiqn clinical trails that will test clinical applicability of our work in leukaemia treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	We will focus our studies on mice. The mouse is the most widely used system to study stem cells. Mouse and human stem cells share similar properties. Availability of reagents and various transgenic mouse strains make them optimal models. We envisage that we will need approximately 20,000 mice to be engaged in breeding over the period of 5 years. In addition, approximately 4,500 mice will undergo stem cell transplantation and in 2,500 mice we will delete genes of interest to assess blood parameters.

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 mice will be used for breeding which involves no adverse effects and severity limit of this procedure is below threshold or mild. In some rare cases we may have to breed healthy mice that are susceptible to leukaemia. These mice will be carefully monitored and scored, and because they may be close to developing leukaemia the severity limit will usually be moderate. However, a very small number of sudden deaths may occur and this would be categorised as severe. In many cases the mice resulting from the breeding procedures will be genetically modified allowing us to determine the functions of the genes of interest. These genes will typically be important for blood production or blood cancer development. As a consequence, the mice may develop mild anaemia or blood cancers. Such mice will be very carefully monitored to detect humane endpoints. Any mice showing unexpected side effects will be culled. Blood sampling or injections cause slight short- lasting discomfort. All mice will be humanely killed at the end of each experiment. The level of severity of these procedures is moderate. The gold standard assay in haematology is the bone marrow or stem cell transplantation. In this procedure, bone marrow from recipient mice is removed by irradiation (to make space for new cells) and the mice are injected with new stem cell cell or bone marrow cells. After irradiation animals may have hunched posture or lose bodyweight. The severity limit of this protocol is moderate. Some of our transplanted mice may develop blood cancers. Mice will be carefully monitored and mice showing unexpected side effects will be culled. All mice will be humanely killed at the end of each experiment. However, a very small percentage of sudden deaths may happen and this would be classified as severe. Finally, in some cases we will need to obtain large number of stem cells from mouse bone marrow. To achieve this we will use a standard protocol under which the mice will be injected with 5-FU, a substance used to treat cancer patients, and killed within a several weeks. We do not expect any adverse effects under this protocol and the severity limit is mild.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have explored the possibility of experimental methods that do not require the use of animals. To replace animals we will use blood-derived cells which can be investigated in culture. In some instances, however, the use of animals is currently unavoidable as many aspects of stem cell and leukaemia biology cannot be simulated using tissue culture. When exposed to culture, stem cells change their properties and therefore these processes have to be studied using animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The most efficient breeding will be applied. The smallest number of mice required will always be used. These numbers are based on our previous experience and power calculations. Assays requiring cells from animals will be optimised to minimise the numbers 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.	If mice are in pain, 'pain killers' will be given. Animals exhibiting any unexpected side effects will be culled. Dosing will be performed using the least invasive route possible, blood sampling will be to a maximum volume unlikely to cause physiological harm, animals with immune incompetency will be barrier-protected to maintain their health and welfare and as required, analgesia will be given. Invasive procedures will be conducted aseptically to reduce the risk of infections.

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<tr><td>Project 21</td><td>Blocking autoimmunity by targeting CD4 T cells</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Autoimmunity, CD4 T cells, Tregs, OX40</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of this project is to test the requirement for two molecules expressed by certain immune cells in the development of autoimmunity (where immune cells attack other cells of the body) in mouse models. Having tested the role of these molecules we will also test whether blocking these signals using reagents equivalent to those that would be used clinically can successfully block autoimmune disease in the mouse models. This will provide direct in vivo evidence that targeting these pathways can inhibit autoimmune responses, paving the way to translate these findings to the clinic.</td></tr>
<tr><td>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)?</td><td>The experiments described here will provide critical in vivo data on the ability of reagents targeting two molecules on T cells to block their ability to cause damage when reacting to self (autoimmunity). Such data is the vital first stage in developing therapies for targeting human autoimmunity. The basic data generated here will provide the initial starting point for subsequent development of human reagents. Given the current problems with autoimmunity developing when trying to improve anti-tumour responses, targeting of pathways that may limit autoimmune responses is of real benefit to patients. Clinical reagents targeting these molecules have been developed by pharmaceutical companies, therefore with robust data from animal models, rapid translation of this work to the clinic is possible. It is vital to robustly test the role for these molecules in vivo to ensure that, if beneficial, such reagents</td></tr>
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	can be used in the correct clinical setting.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 16,000 mice will be required to breed and maintain colonies of the mice and perform the planned experiments over the five year time 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 experiments described here will reach moderate severity in some cases due to the autoimmune disease models used or through the generation of bone marrow chimeras to precisely dissect the cell type responsible. To assess the signals required for autoimmunity, it is inevitable that autoimmunity must develop in some animals to provide the model in which the response can be tested. Animals developing autoimmunity will be carefully assessed and suffering will be kept to a minimum. All mice will be killed humanely at the end of the protocol or should clinical end points be reached, then prior to the end of the protocol. In the course of these experiments, animals will also necessarily be subjected to injections, oral dosing, blood sampling and/or modification of their diet. In all cases adverse effects will be minimised by the use of the most refined techniques by skilled staff, and humane endpoints have been predefined.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project requires animals as autoimmune responses must be analysed within live animals rather than in a test tube to accurately model the complex dynamics of real immune responses. Results using non-animal alternatives would require subsequent experiments in animal models before pre-clinical work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will consult with a statistician and use the minimum number of mice needed to ensure statistical significance. We have substantial experience in mouse models of immune responses and have published extensively, providing clear frameworks within which high quality publishable experiments will be performed.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Mice are an excellent model for the human immune system and have been extensively characterised and validated. These animals provide the best means for analysis given the wealth of reagents available and the wide range of genetically modified

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	mice. Using genetically modified mice, precise mechanisms can be tested, facilitating the development of therapies for human use. The methods described are established in the lab and every effort has been made to develop refined techniques causing minimal clinical side effects, Examples of refinements include: - use of attenuated Listeria monocytogenes strain has reduced the need for adjuvants which can cause local inflammation. - when we immunise mice, injections under the skin at the base of the front paw pads target the draining lymph nodes without needing to inject directly into the footpad which can cause swelling at this site and problems with walking on that limb. - rather than provide tamoxifen (a drug used in some of the mouse models to induce gene expression) by repeated injection, food containing tamoxifen is used.

Project 22	**Phagocyte function and inflammatory bowel disease study**	
Key Words (max. 5 words)	Bacteria, Crohn's disease, neutrophil, granulomatous	
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)	Your Immune system defends you from microbial challenge as well as detects and removes abnormal cells from your body. If the immune system becomes damaged through the inheritance of a defective gene or exposure to an infective/chemical agent you are at an increased risk of developing life threatening diseases and cancers. Our research is focused on understanding how the immune system works in relation to the killing and eradication of microbes and how these processes go wrong in human disease, specifically Crohn's disease and ulcerative colitis. It is currently unclear why people develop Crohn's disease and ulcerative colitis and the precise mechanisms responsible remain elusive. A number of large studies have recently been performed and over 160 genes identified which have a potential role in disease progression. In order to study the individual genes and attribute them to specific functional roles numerous new studies have to be performed. One of the most informative studies will involve the development and study of genetically altered animals which replicate the findings in Crohn's disease and ulcerative colitis patients. Our study focuses on a number of genes we discovered as abnormal in immune cells from patients. Our major objective is to identify the role these genes play in the immune system and how they function in the bowel. We will also investigate how the immune system deals with microbes and the detailed	

	mechanisms of how microbes are killed.
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 delineation of the mechanisms underlying microbial killing could lead to the development of drugs that increase the efficacy of this process, helping design new treatments and therapies for individuals with immune dysfunction. Knowledge of the molecular mechanisms of Crohn's disease and ulcerative colitis could result in the development of novel diagnostic methods and new therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice in this study, and envisage using approximately 5,000 during the next 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?	All proposed experiments are well established protocols and no undue adverse side effects are expected. All experiments will be monitored as directed by the protocol and any adverse event will be dealt with immediately. The most common side effect is bloody diarrhoea. The major adverse effect will result from development of blood poisoning through the administration of microbes and microbial products. Blood poisoning will develop rapidly and will be identified through regular monitoring in the first hour post procedure. The animal will either be immediately culled using a Home Office approved Schedule 1 method or the Named Veterinary Surgeon consulted and advice sought.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible, cells from sources other than laboratory animals will be used. These include appropriate cell lines, or experiments are performed directly on volunteer patients with known diseases and the appropriate healthy control individuals. Animal experimentation is only used when there is no appropriate human equivalent or where it would be impractical to study humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Initial doses of any test substances or organisms will be determined through literature search. Pilot studies will be performed with low and then increasing doses of substances or organisms for each route of inoculation, starting at a low baseline. Once the dose required to produce a clinically identifiable response has been established, the groups of animals will be

	increased to numbers which provide sufficient power to enable identification of any significant differences.
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 will be used because they are the standard mammalian animal model for gene targeting technology and most of our experiments will be performed on genetically modified animals. Our methods have been chosen that most closely resemble the human diseases we are interested in. All methods used in these studies use standard protocols which have been published. Animal welfare is paramount at all times. Animals will be checked daily or more frequently if required during experiments, and if animals demonstrate signs of undue sufferings, they will be humanely culled, by Home Office approved methods (Schedule 1).

Project 23	**EGFR mediated modulation of immune responses**	
Key Words (max. 5 words)	inflammation, immune regulation, tissue remodelling, protection against pathogens and wound repair	
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)	Our research is focused on the function of specific growth factors, the so called “Epidermal Growth Factors”, that are expressed by cells of the immune system and that play a central role in wound repair and the regulation of immune responses. We have discovered that the immune system has adopted two of these growth factors that are of central importance for the development of tissues and organs. Under inflammatory conditions, cells of the immune system, so called leukocytes, re-express either of two of these growth factors that otherwise are only expressed during embryo-development. These two growth factors bind the same receptor, but exert opposite functions. Many diseases and disorders, such as allergies and asthma, many cardiovascular as well as fibrotic diseases, are caused by the immune system and can currently not efficiently be treated. Many diseases develop, when inflammation is not properly controlled. Long lasting, strongly inflammatory conditions can lead to auto-immune diseases, such as arthritis or diabetes, as well as to cardiovascular diseases. Long lasting, weakly inflammatory conditions, on the other hand, can induce an immune-suppressive environment, such as for instance within tumours, and can lead to fibrotic diseases. Both of our growth factors play a pivotal role in either of these opposing processes.	

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 objective of our research is to determine i) which cell types produce either of the two growth factors, and under which inflammatory conditions (strong inflammatory versus mild inflammatory); ii) how the two growth factors contribute to disease causing (pathological) developments within inflamed tissues; iii) how the expression of the two growth factors influences the functioning of the immune response at the site of inflammation. Based on this knowledge we will iv) develop tools to either inhibit the function of either of these two growth factors (synthetic inhibitors) or directly target one of these two growth factors to the site of inflammation (chimeric antibodies) or to specific cell types (bi-specific antibodies). These novel tools will then be tested for their therapeutic capacities in mouse models of human diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	This set of experiments will provide a deeper understanding of how inflammation regulates immune responses and disease-causing processes. We expect — and have already demonstrated in the past — that novel tools that allow the manipulation of the function of these two growth factors will allow us to tilt the state of inflammation in favourable directions. In this way we believe that many immune- mediated diseases could efficiently be treated and potentially even be cured, as we have demonstrated before in animal models.
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?	Our experiments involve the use of genetically manipulated mice and several backcrosses of differently manipulated mice. Approximately 20000, animals will be used over the 5 year period of which the majority of animals will breed and maintain genetically altered mice.
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 a highly complicated network of different cell types that at different time points secrete distinct sets of factors that all influence each other and finally enable efficient clearance of infections while maintaining tissue integrity. While in the last decades we have made tremendous progress in understanding the underlying processes of this system, we are far away from being able to replicating the immune system in cell cultures or “in

	silico” with computer animations. Our specific studies rely on looking at immune responses in the context of the whole body. In some experiments we are inducing chronic inflammation. Such immune responses often change fundamentally over the course of the experiment and, at times, these processes can involve multiple body systems. Thus, to be able to address the raised scientific questions and to develop and test novel therapeutic tools, mouse experiments will have to be performed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always aim at using the minimal number of mice that will give us clear and reliable answers. If our animal groups are too small, we may have to repeat the experiment to ensure that the result we got was actually meaningful. Therefore, prior to the initiation of each experiment, a power calculation is performed that determines the number of animals that most likely will be necessary to obtain a valuable answer and to achieve statistically significant differences between experimental and control groups. This power calculation is normally based on results derived from previous experiments or from literature. The numbers per group are regularly re-assessed when more data are available for input into our power calculations. Also our breeding colonies are kept to a minimum.
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 represent the most tractable and appropriate species for our in vivo studies given the availability of transgenic strains, reliable disease models and commercial reagents. In the last decades, mouse models have been developed that very closely resemble those of human diseases. Thus, the most physiological relevant system can be studied and the underlying reasons for the development of specific diseases can be determined. Also our therapeutic interventions can be performed in the most relevant setting. This will then ensure the most efficient way to translate our findings into a clinical setting.

Project 24	**Modulators of Immunity and Inflammation**	
Key Words (max. 5 words)	Infection, therapy, autoimmunity, allergy, cancer	
Expected duration of the project (yrs)	Five	
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)	To identify new potential drugs and other molecules from helminth parasites, to test them individually and in combination for their potency as vaccines and as immunomodulators. We will then establish the mechanism by which these products influence the host immune response, and to assess their value as therapies in diseases in which the immune system overreacts to harmless substances such as in allergy, autoimmunity, organ transplant rejection, and inflammatory bowel disease. We will thereby identify therapeutic effects at the molecular level, to separate the harmful from beneficial elements of parasites, and translating findings into future pharmaceutical products. The project also encompasses new and exciting directions that investigate whether helminths and their products can alleviate metabolic disorders (such as obesity and type II diabetes), and whether it is possible that they may also be the source of new treatments to combat 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 benefits of discovering new, natural, mediators that parasites have evolved to dampen host immunity include new therapies for many of the ”Diseases of Modernity” that are increasingly prevalent in the Western world. In addition, new immunological strategies (including vaccines) will lead to treatment and elimination of human helminth parasite infections in tropical countries, and similar infections in livestock across the world including the UK.	

What species and approximate numbers of animals do you expect to use over what period of time?	Mice, rats, jirds and rabbits. Almost all the animals used will be mice, with an expected total of 35,705 including a large proportion of breeding colonies.
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 the animals will experience mild or moderate levels of severity. A small proportion of those involved in experiments of major human diseases such as multiple sclerosis and inflammatory bowel disease may develop more severe conditions but will be promptly euthanised. In other studies such as on airway allergy, exposure of mice to innocuous allergens is not harmful in itself, and the only discomfort is at the end stage of airway allergy measurement. Similarly, short term tumour growth will be employed in cancer models of mice to stimulate specialised host cell populations, but tumours will not be allowed to progress beyond a moderate endpoint. All animals will be humanely euthanised at the end of the work.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The only means of assessing immune mediated diseases and factors which regulate functional immunity in affected tissues and organs is by the use of animals. For example, allergy, autoimmunity, organ transplant rejection, reperfusion injury and inflammatory bowel disease are immunopathologies which become manifest as a result of dysfunctional over-reactivity of the entire immune system. Our research in vitro indicates many promising approaches for the treatment of these diseases, but the complexity of the in vivo network of tissues, cells and mediators requires research in live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are increasingly precise in their use of specific genes (and animals lacking those genes), and our laboratory assays increasingly high throughput permitting us to capture the maximum data from each study. Hence fewer animals can yield more information than was previously possible. In addition, within each experiment, we use the minimum number of animals that will provide significant results, given the expected differences between groups, as deduced by power calculations. Group sizes are re-assessed in the light of new data to strive to minimise numbers of animals required.
3. Refinement	For studies of immunological pathways, and for

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.	allergy, autoimmunity, transplant rejection and inflammatory bowel disease studies, the mouse is the most appropriate species, because of the wealth of information and range of reagents now available. We have chosen models of allergy, autoimmunity and inflammatory bowel disease which provide minimum suffering and minimum duration respectively. Within these disease settings, we also prioritise the most refined models with early indicators yielding data before harm becomes severe, rather than more rapid and acute models in which multiple pathologies may confound analysis. While most studies involve the mouse, some work requires rabbits (as blood donors, with no significant harm), rats (for parasite infections and generation of antibodies, with minimal harm) and jirds (for parasite infections), in each case being the most appropriate or the only possible species available for the studies involved.

Project 25	**The role of B cells and microbiota in autoimmunity**	
Key Words (max. 5 words)	Autoimmunity, B cells, gut-bacteria.	
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)	Diseases in which the body's own immune system 'attacks' the body's cells and organs are called "autoimmune diseases". In a number of diseases, cells of the immune system that are supposed to prevent this autoimmunity are dysfunctional. The objective of this project is to gain understanding of what makes particular cells dysfunctional in autoimmunity, with a specific focus on those B cells that suppress inflammation. We also hope to gain insight into how the bacteria that colonize the gut influence the development of autoimmune disease, or whether certain bacteria protect 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)?	The short-term benefits of this licence are, first of all, that our results will increase the understanding of how B cells of the immune system develop throughout autoimmunity, and enhance the understanding of how the presence of specific bacteria may influence the development and severity of autoimmune disease. This work, which we hope to publish in peer-reviewed journals and report at conferences, will also be beneficial to the work of scientists in a number of disciplines, including immunology, cancer, and transplantation, conditions that are all also thought to be influenced by the cells we are studying. Overall, our long-term aim is gain insight into the biological processes underpinning autoimmune disease in such a way that we can find new targets for treating these diseases, or so that we	

	can refine existing treatments. Greater understanding of the factors influencing the development of autoimmunity may lead to the development of new, less-expensive treatments, leading to better long-term prognosis and less economic burden on the NHS.
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use mice in this project licence. We have estimated that we will use approximately 11,000 mice over the period of 5 years. This will allow us to assess how specific protective immune system cells and bacteria influence disease in both spontaneous and induced models of rheumatoid arthritis, lupus and multiple sclerosis.
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?	Experimental procedures carried out on our mice can be broadly divided into two categories: inducible-models of autoimmune disease and spontaneous models of autoimmune disease. Mice used under these protocols will experience a moderate level of severity at most, and many steps are in place to prevent prolonged suffering or the development of a high level of severity. As the models we are using reflect human arthritic diseases, the adverse effects will include some inflammation, some weight loss, and well as some pain at inflamed sites. At the end of the experiment we obtain clinical data and tissue to look at the response of the immune system to inflammation.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Autoimmune disease is the result of a complex interaction between multiple organ systems and multiple immune system cell types. Thus, unfortunately, at present, we are unable to model autoimmune disease using isolated cells from humans or healthy animals, or cell-lines in a test tube. The data and results obtained from experiments where we look at the kidneys, liver, spleen, blood, lymph nodes and joints of animals with disease are more representative of human disease. Kidneys will be taken from mice with lupus; joints will be taken from arthritic mice; liver, spleen, blood and lymph nodes will be taken from every mouse to analyse the immune response. We must have these results if we wish to develop new therapies for human patients. However, where possible we use cell lines or samples taken from humans instead.
2. Reduction	Careful consideration is given to calculate the

Explain how you will assure the use of minimum numbers of animals	minimum number of mice needed to give statistically significant results. The size of experimental groups has been decided after consultation with the statistician at our institute and we will continue to consult with statisticians for optimization of the experiments throughout the life of the licence.
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 in our experiments as these animals have well characterized immune systems that share many similarities to humans. Research with mice has been fundamental to the development of many drugs that are used to treat immune system related diseases. In our research we will start by using inducible models of disease that result in short-term, highly reproducible disease with almost 100% reliability. The reproducibility and reliability of the mice getting disease will allow us to use fewer mice. Following identification of biological processes that may be involved in disease development, we will look at these pathways in spontaneous mouse models of autoimmunity that are more translationally relevant to human autoimmune disease, which is also spontaneous. In general, to minimise welfare harm, we inspect the mice daily and administer pain relief where possible.

Project 26	**Evaluating Candidate HIV/AIDS Vaccine Strategies**	
Key Words (max. 5 words)	HIV, Vaccines, Pathogenesis	
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)	In spite of 30 years of intensive research, the world does not know the scientific basis of making an effective vaccine against HIV/AIDS. As a result we do not know whether a vaccine needs to generate antibodies that eliminate the virus particles, killer T cells that identify HIV infected cells in the body and prevent them from producing more virus or some other type of vaccine response. Before a novel vaccine can be tested on humans in clinical trials, it is essential that evidence that it can control virus infection is gathered from appropriate animal models. At our Institution, we collaborate with academic scientists and clinicians performing vaccine trials to facilitate the development and evaluation of candidate vaccines that will prevent the transmission of HIV. We do this by applying animal systems that best model the responses made in man to vaccination and models that mimic infection with HIV. In the next 5 years we will: a) Collaborate with scientists who are modifying the HIV coat protein to try and make it a better vaccine for generating large amounts of antibody that can neutralise the virus and prevent infection taking off. b) Build on our previous research to investigate how to make better antibodies to the virus coat that can neutralise the virus when the antibodies are present	

	at lower concentrations in the blood. c) Collaborate with an international consortium that will investigate how drugs called microbicides that can prevent HIV infection for a short period can be combined with vaccines to be most effective together. d) Build on our previous work to investigate how a completely novel vaccine works. This vaccine gives very good protection but it cannot be tested in the clinic because of identified risks in certain populations. Our goal is to understand how the vaccine works so a new safer vaccine can be made that protects in an identical manner. e) Use samples that arise from the vaccine studies above to understand how infection with HIV can cause neurocognitive problems even when drugs are controlling the infection process.
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 arising from this work will be: • the development of better vaccines that not only generate more antibodies against the virus but also make more potent antibodies that block infection more effectively • the identification of the best combination of vaccine and anti-retroviral drug to progress into clinical evaluation • identify a completely novel approach to making an effective AIDS vaccine • Better treatments that will prevent HIV infection leading to neurocognitive problems later in life.
What species and approximate numbers of animals do you expect to use over what period of time?	Across the 5 years of the project we anticipate requiring: Mice: 50 Rabbits:50 Guinea pigs: 40 Cynomolgus macaques: 100 Rhesus macaques: 50
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?	Rabbits, guinea pigs and mice will be immunised with vaccines with side effects very similar to those used in humans. The injection of vaccines or other substances will confer only temporary mild side effects. Collecting blood samples from superficial veins may cause bruising which should resolve quickly. Occasional minor surgery to obtain peripheral lymph nodes whilst under anaesthesia may cause temporary soreness at the site that will be

	dealt with analgesics. At the end of the studies the subjects will be killed humanely Some macaques will be bled only. This will be performed under anaesthesia. This may cause bruising which should resolve quickly. Some macaques will receive vaccines delivered under the skin or in muscles, or intranasally. These will cause only transient discomfort. Macaques will be inoculated with infectious virus that could cause AIDS-like disease. Any infections will be monitored closely by taking regular blood samples and animals will be weighed to investigate disease associated weight-loss. Our experience is that within the duration of studies minimal deviation from good veterinary health is observed. At the end of these studies where immunisation and virus challenge is involved, all macaques will be killed humanely by overdose of anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At the present time, it is not possible to evaluate the ability of vaccines to elicit immune responses through *in vitro* assays alone. Moreover, since for HIV, we do not know the types of vaccine responses that are required to prevent transmission of the virus, we require an animal model which can be deliberately exposed to an AIDS virus once the vaccinations have been completed. For these studies we need a model system where an appropriate AIDS virus exists
2. Reduction Explain how you will assure the use of minimum numbers of animals	In all studies, we seek professional bio-statistical advice to ensure that experimental studies performed use the fewest subjects capable of providing statistically significant data We shall also ensure that all samples collected will be used to undertake multiple measures of the immune response or viral infection maximising data generated.
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 our studies where we wish to characterise the ability of antigens to generate antibodies and killer T cell responses (project 1), we propose to use laboratory mice, rabbits or guinea pigs. Which species is selected depends on the types of biochemical and immunological tests we need to undertake in the laboratory using materials collected from the animals. To minimise the welfare cost to the animals in these studies they are kept as short as possible and the most in depth sampling is performed only after the subject is killed humanely. For projects 2, 3, 4 it is essential that they are

	performed in laboratory purpose bred macaques. This is because the types of responses to the vaccines are known only to occur in humans and non-human primates because of the close evolutionary relationship. In other studies, we require the subject to be vaccinated and then exposed to an AIDS virus to establish whether the vaccine works. Only humans and non-human primates are susceptible to this family of viruses. If animals become infected then we keep a very close watch on the animals to look for any symptoms of AIDS. Our previous experience means that we usually complete our studies well before symptoms are likely to develop. At the end of the study the subjects are killed humanely.

<table>
<tr><td>Project 27</td><td colspan="3">Molecular Networks of Inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Immunology, Inflammation, Autoimmunity, Signalling</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The innate immune system is our first line of defence against infectious agents such as bacteria and viruses. If, however, the activation of innate immunity is prolonged or inflammation fails to resolve after it has served its purpose, it can lead to the development of chronic inflammatory and autoimmune disorders such as rheumatoid arthritis and psoriasis. The aim of my research programme is to understand in molecular detail the intracellular networks limiting the innate immune response and promoting the resolution of inflammation in order to identify new drug targets for the treatment of inflammatory diseases. We will then identify the best strategy for disrupting the function of the signalling molecules and test their therapeutic potential in pre-clinical models of human disease.</td></tr>
<tr><td>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)?</td><td colspan="3">The research programme will provide two major benefits. In the short-term, it will advance our knowledge of the molecular mechanisms controlling the innate immune system and promoting the resolution of inflammation. This information is essential to understand how our body fights infection at the molecular level and to identify the reasons that chronic inflammatory and autoimmune diseases develop in certain patients and identify strategies to correct these problems. In the long-term, it has the potential of improving human health by identifying new drug targets for the treatment of chronic inflammatory and autoimmune disorders including rheumatoid arthritis, which remain a group of debilitating diseases without any cures. Indeed, we</td></tr>
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	have already identified such a target and we will be investigating the effect of inhibiting the enzymatic activity of this family of proteins on the development of inflammation as part of this programme. These experiments will provide the experimental evidence necessary to develop strategic partnerships as a first step to the elaboration of new therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used for this research programme. I estimate that we will need 15000 over the course of the 5 year licence. Most of these animals will be used for breeding purposes with only a small fraction being needed for studies in live animals.
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 animals will be used for the maintenance of colonies of genetically-modified mice which display no overt phenotypes. A small number of animals used in the research programme may have genotype-related adverse phenotypes often characterized by the progressive development of inflammatory disorders. These animals will be maintained on a moderate severity limit and several measures are in place to minimize the suffering of these mice including additional monitoring of the animals, treatment regimes, and establishing age limits. To model inflammatory diseases, we will use animal experimental approaches accepted in the field with early endpoints to avoid unnecessary suffering. Where possible, non-invasive monitoring technologies will be used in place of invasive methods. At the end of all studies, animals will be terminated using an approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals will be used to establish primary cell culture systems from wild-type and genetically-modified mice to interrogate the effect of interfering with selected signalling molecules. Subsequently, animals will be needed to evaluate new therapeutic strategies in preclinical models of human disease. Unfortunately, immortalized cell lines respond very differently to inflammatory stimuli compared to primary cells and the immune response is a dynamic multi-cellular process that cannot be accurately recapitulated ex-vivo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Most of the experimental studies will be performed using primary cells isolated from animals, which will reduce the number of experiments performed on live animals. Breeding strategies and sample processing

	methodologies will be optimized to maximize information from as few animals as possible. The minimum number of animals required in each experimental group to obtain scientifically and statistically relevant data will be determined using statistical methods in combination with experience in the field.
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 transgenesis allows the creation of animals with specific mutations to probe the (patho)physiological function of signalling molecules. The power of mouse genetics in combination with mice being the least sentient species in which inflammatory models have been developed make it the ideal species for this research programme. Most genetically-modified mice present no significant phenotype deviating from the norm. In the case of mouse lines where animals require additional attention, a plan will be developed in consultation of the named vet to minimize suffering of these animals. For in vivo experiments, models have been selected to represent the most up-to-date refined protocols accepted by the scientific community. Every effort will be made to reduce suffering by implementing measures to this effect including daily monitoring and welfare scoring system.

Project 28	**Production of Blood Products**	
Key Words (max. 5 words)	Animal infection antisera	
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
	x	Regulatory use and routine production
	x	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 objective of this project is to produce antibodies to viruses or bacteria, from animal blood. Antibodies specific to one virus or bacterium is produced in animal blood when that animal is infected with that virus or bacterium. This blood can then be used to help determine whether other animals are also infected. The product is also used to check that animals have been successfully vaccinated, and to check the success of new 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 benefits are to enable breeders of laboratory animals and breeders of turkeys and chickens to make sure their animals are infection – free. Also, the benefits are to produce vaccines to human infections and even possibly a treatment for cancer.	
What species and approximate numbers of animals do you expect to use over what period of time?	Typically we would use around 300 mice a year, but could also use a small number of rats, hamsters, guinea pigs, rabbits, chicken or ducks.	
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?	During their time with us, the animals lead a much uninterrupted caged lifestyle. Some animals may have a blood sample taken from them – but this only causes brief discomfort. The final product is the antibodies in the blood which fight infection. Blood samples may be taken to determine when the animals have produced a high enough level of antibody. When a suitable level of antibody has developed, the animals are given an anaesthetic from	

	which they will not recover and their blood is collected as a source of antibodies. The animals are not expected to experience severity greater than what is regarded as mild. Guinea pigs are also used to provide red blood cells. Following the administration of an anaesthetic from which they will not recover their blood is collected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no appropriate alternative in this case to this sort of test, called serology, for the screening for these viruses and bacteria. There are other tests, but this is best for checking a large number of samples efficiently.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Supply of this product is to meet customer demand only, and is diluted as much as possible to make it do as many tests as possible.
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.	Antisera is diluted as much as possible to make it go as far as possible, and as few animals are used as possible. The type and dose of injection given to the animal is under constant scrutiny to make sure we cause as little distress to the animal as possible.

Project 29	**Pathophysiology of autoimmune diseases**	
Key Words (max. 5 words)	Autoimmune disease, immune molecules	
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
	√	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 specific research aim of this project is to understand the functions of molecules in the development of autoimmune diseases such as multiple sclerosis (MS) and uveoretinitis, and identify whether these molecules are potential therapeutic reagents or targets for treatment. The ultimate aim thus is to assist in the development of novel therapies to treat human autoimmune 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)?	Autoimmune diseases occur in patients when their immune system attacks their own tissues. For example, uveoretinitis and multiple sclerosis (MS) occur when the immune system attacks the retinal tissues and central nervous system tissues respectively. Autoimmune diseases comprise more than 50 distinct diseases and syndromes such as rheumatoid arthritis and type 1 diabetes, and affect about 5% of the population in Europe and North America. The total societal disease burden for autoimmune disorders is difficult to estimate, however autoimmune disorders as a group are among the most expensive diseases faced by society today. The data from this project will add to the current knowledge of immune mechanisms in the development of MS and uveoretinitis, thus help us to understand better how neurological autoimmune	

	diseases such as MS and uveoretinitis develop. In the long term, it will lead to the identification of mediators, which cause the disease as well as those capable of preventing the disease. Based on this knowledge, further novel rational therapeutic strategies for patients with autoimmune diseases will be developed, all of which have enormous impact on the economy and health care of humans.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use well-established research mouse models: experimental autoimmune encephalomyelitis (EAE) and experimental autoimmune uveoretinitis (EAU) for human multiple sclerosis and uveoretinitis study respectively. We estimate that about 2500 mice will be used for EAE study and 1000 mice for EAU study in the next 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?	To understand the functions of specific genes and molecules in the development of EAE and EAU, genetically modified animals will be bred and maintained. Genetic modification might compromise mice health, but in general the adverse effects should be mild, e.g. thinner bones have been observed in ST2 deficient mice, however no clinical abnormalities have been reported. Most likely animals will be used to understand immune mechanisms of uveitis and MS disease. We will induce EAE and investigate the effect of new molecules and potential reagents in MS disease development. The main adverse effects will be the development of limb paralysis. The severity category of the protocol is severe, however not all mice immunised for EAE will fall into the category (e.g. experiments are designed to study the early immune response in EAE mice). As soon as disease progresses to the point necessary for scientific analysis, animals will be humanely killed. We will induce EAU to study the effect of new molecules and potential reagents in uveitis disease development. The severity category of the protocol is moderate, i.e. immunised mice have no abnormal behaviour or weight change despite inflammation in their eye. Again as soon as disease progresses to the point necessary for scientific analysis, animals will be humanely killed for research purpose. There may be other general adverse effects associated with anaesthesia, administration of

	reagents, all very rare in our experience.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our research will involve a significant amount of *in vitro* laboratory work, using tissue culture techniques. However work in tissue culture cell lines can never have the physiological relevance of the *in vivo* work and there is no *in vitro* model that can reproduce the complex interplay of molecules and cells among different tissues and organs that shape the mammalian immune system. To investigate the function of molecules in the development of autoimmune disease multiple sclerosis, a disease in the central nervous system (CNS), the research requires an intact immune system, CNS, and the complex cell and molecule interaction network in vivo. This can only be done using an animal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers used will be reduced through ensuring efficient colony management and careful experimental design generating statistically useful data with the minimum number of individuals. The retinal imaging technique and clinical scoring methods for retinal inflammation allow early stage disease to be investigated without killing the animals, this will reduce the overall numbers of animals required. Furthermore, we will plan our experiments very carefully, harvest multiple organs from the same animal (e.g. spleen and lymph nodes to be used for immune response analysis, central nervous system for tissue inflammation analysis). This will reduce the overall number of animals required for the project.
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 experiments are mostly short-term and are kept to the minimum length needed for the experiment. We use clinical scoring systems for EAE mice, which reduces animal suffering and inform on early endpoints. Also we will consider the use of the least invasive methods to give substances in any of project plans.

Project 30	**Investigating the immune response to somatic corneal stem cell allografts**	
Key Words (max. 5 words)	Cornea, stem cell, immune system, transplantation, graft rejection.	
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)	Over the past 10 years, several hundred patients have received stem cell transplants to their cornea as part of clinical trials. In trials where the transplanted stem cells were taken from the patient's other healthy eye the treatment worked very well. However, in trials where the cells were taken from unrelated donor eyes the treatment was rarely successful. The reason for this is believed to be that the body's immune system rejects corneal stem cell transplants taken from unrelated donors. The objectives of this project are to improve our understanding of how and why the immune system rejects non-self donor stem corneal stem cell transplants and, once we have identified the key steps in this process, to test different methods of preventing such rejection so that the body's immune system learns to accept and become tolerant of the transplanted non-self donor stem cells.	
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 human trials of corneal stem cell transplants, the cells that are transplanted can either be taken from the patients other eye if it is healthy or if both eyes are diseased then donor cells can be taken from a deceased human donor. The outcomes of non-self donor cell transplants in patients with bilateral eye disease are very poor when compared to transplants using the patient's own cells from their fellow healthy eye. The reason for this is believed to be that the body's immune system rejects the non-self cells.	

	Corneal opacification is the 4th most common cause of blindness in the world (WHO statistics). Up to 250 new patients per year in the UK are diagnosed diseases that result in limbal stem cell deficiency and that have no prospect of a permanent cure using any existing treatment other than stem cell technology. The clinical prognosis for non-self donor stem cell transplants in such patients is extremely poor. At present little is known about the interaction between corneal stem cell transplants and the immune system in these patients. It is for this reason that we have sought to undertake this project and investigate this problem. This project should provide new knowledge and insights into this area of biology. If successful the work will benefit patients both indirectly through new scientific knowledge regarding their condition and its treatment, and directly through translation of our findings via future clinical trials..
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to 2500 wild type mice. We will also breed and use up to 2000 genetically altered mice. Genetically altered mice will either contain modified genes that enable us to gather information about the stem cells themselves (such as whether the cells survive the transplantation process) or genes which modify different aspects of the immune response to a transplant (such as genes that prevent signalling between immune cells). The project will take 5 years to complete.
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 breeding of genetically altered mice is not envisaged to cause any adverse effects. The mice we use should not experience any major observable abnormalities in appearance or behaviour from their non-genetically altered counterparts. Some mice will have weakened immune systems and therefore will be prone to infections but measures will be taken to protect these mice from being exposed to or developing such infections. We propose to perform stem cell transplants in mice and to investigate the rejection of such transplants by the immune system. We will begin by performing a series of pilot experiments to establish the most successful way of transplanting corneal stem cells into the mouse cornea. In order to allow donor stem cells to take up residence in the cornea of the recipient mice it may be necessary to perform a separate surgical procedure on the eye prior to transplantation of corneal stem cells. This preparatory

	step will involve removing the mouse's own corneal stem cells using an alcohol solution and surgical removal of tissue. The likely adverse effects of both of these procedures are pain (which will be treated by keeping the eye closed using a stitch, using eye-drops and pain relieving medication) and blurring of vision in the operated eye only. Infection of the cornea is a potential but unintended adverse effect. This will be prevented by administering antiseptic drops prior to any eye procedures and antibiotic drops following eye procedures. Once we have established how to successfully transplant stem cells into the mouse cornea we will set about investigating how and why the immune system rejects these cells and how this can be prevented. We will perform corneal stem cell transplants from non-self donors and will examine the mice under general anaesthesia up to 3 times per week to evaluate their eyes and take scans to study. We will take blood samples and samples of fluid from inside the eye to look for the signals of immune rejection. This aspect of the work will result in transient pain but will not result in lasting harm for the mice. Preventing the immune rejection process will require the administration of certain drugs or other molecules either by injection or orally. These drugs may have toxic side effects but we will perform toxicity testing in the laboratory prior to using them in live animals and will test small doses in single animals prior to proceeding to any wider use. Animals will be studied for the minimal possible duration post surgery and for no longer than 9 months at most. They will then be humanely killed. If any animal is found to be in pain or distress or to have exceeded the severity limits we have put in place it will be humanely killed regardless of whether or not the experiment has been completed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The rejection of donor cells is a complex process involving many different organs and cell types acting simultaneously at various different locations around the body. Whilst some individual parts of this process can sometimes be replicated in a laboratory dish it is impossible to investigate the workings of the immune system as a whole without a living model. Where possible we will use laboratory dish-based assays to

	answer specific questions but the use of living animals will still be necessary.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where we must use animals, each one will be used in most effective way possible. For example the numbers of mice we use as donors of tissue will be minimised by coordinating experiments so that one donor mouse will provide spleen cells for one experiments, bone marrow for another experiment, and corneal tissue for a third experiment. We will further reduce the number of animals used by ensuring the highest possible standard of experimental design. This will ensure that need for using sufficient animals to make the results scientifically valid and useful is balanced against the need to avoid unnecessary use of any more animals than are required to answer the specific research question that we are asking. The experimental group sizes needed will be reduced by using inbred mouse strains where possible, block study design with 2-way ANOVA analysis of the results, post hoc analysis of the results (Dunnett's test and Bonferoni correction). Power analysis and sample sizes will be calculated using the resource equation method. **We will also** follow NC3R guidance on the minimising the use of genetically altered mice (https://www.nc3rs.org.uk/minimising-use-ga-mice).
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.	Choice of species: Mice are the lowest vertebrate group in which the immune system and corneal stem cells have been well characterised and which provides the range of genetically altered animals we need to be able to manipulate the immune system genetically and to track cells types. The molecules and biological tools needed to perform this research have been designed for use with mouse immune cells and are not available in any lower species. Recognition of donor cells by the immune system as being genetically different is the fundamental basis of transplant rejection in both humans and mice. By using a mouse model of donor corneal transplant rejection we can closely replicate the clinical setting in humans and learn how best to prevent donor corneal stem cell transplant rejection in human patients. **Surgical technique:** In order to ensure that surgical procedures can be performed in a safe and reproducible manner a training and supervision system is in place to ensure that only people fully

	trained in performing corneal surgery on mice will undertake these procedures. All surgery will be performed under strict aseptic conditions. **Minimising harms:** All research undertaken will be continuously refined to involve a minimum of suffering. Anaesthetics, painkilling medication and soothing eye-drops will be used to minimise discomfort. Antibiotic eye drops will be used at the end of surgery to reduce the chance of eye infections. Animals will be assessed daily to check for any signs of distress or suffering. Where such distress is unexpected and cannot be alleviated the animal will be humanely killed. The cumulative effects of the procedures we plan to undertake have been considered and experiments will be designed to minimise the effect of cumulative harms.

Project 31	**Impacts of urban development on avian immune systems and disease**	
Key Words (max. 5 words)	Immune response; blackbirds; Turdus merula; urbanisation	
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
		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 objective of this project is to test if urban development influences i) avian immune response systems, ii) stress indicators and iii) parasites/disease. It will also provide evidence regarding the associations between these three factors. This is a rapidly emerging research field, and very little data are available to assess the impacts of urban development on wildlife immune systems, stress and 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)?	Towns and cities are perhaps the most extreme example of environments that are highly modified by humans. Globally, the extent and magnitude of urbanisation are predicted to increase markedly in the next few decades. Understanding how the pressures associated with urban development influences production of hormones associated with stress responses, immune function and ultimately disease burdens is important for quantifying the impacts of urban development on wildlife populations. This will be the first study of any vertebrate to investigate simultaneously the associations between stress hormones, immune function and disease in response to urban development. The benefit of advancement in our understanding of fundamental science can be contrasted with the mild nature of the regulated procedure and the absence of adverse effects on the study animals. Thus, a harm-benefit analysis of this proposal would weigh heavily in	

	favour of the potential beneficial outputs.
What species and approximate numbers of animals do you expect to use over what period of time?	Blackbird Turdus merula c. 80 individuals in total over 2 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 protocols require a mild procedure that entails short- term capture of wild birds and that has no adverse effects on the animals in the long term. All animals subject to regulated procedures will be released to the wild following assessment of their wellbeing.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no non-animal alternatives that could be used to address the objectives of this project. In addition, our current knowledge of the study species makes it ideally suited to achieving the project's aims.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It will be necessary to obtain samples from 40 individuals in each study population (urban and rural) to ensure that statistically robust conclusions can be drawn, but each individual will be sampled on a single occasion.
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.	This project involves the use of a single bird species, the blackbird Turdus merula. This body size of this species means that a sufficient quantity of blood can be taken for all the required analyses without causing any harm to the sampled individual. Blackbirds are common in the UK, have a stable population size, and have become a model species in the study of urbanisation impacts.

<table>
<tr><td>Project 32</td><td>An ecological approach to infection and immunity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ecology, coinfection, immunity, helminths, wild mice</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project aims to better understand how parasite infection and an individual’s immune response to that infection affect the health of humans, domestic and wild animal populations. This is important because infectious diseases still cause significant human and animal mortality, and yet while we have good models of disease in controlled laboratory situations, we have much less knowledge about how ecological factors, such as seasonal variation, hosts differences in ages, condition, parasite infection and coinfection affect the consequences of disease and the development of an appropriate immune response in wild animals.

This project aims to incorporate the ecological variation found in natural populations to determine how these factors affect infection and immunity. To do this we will use experiments in wild rodents to understand the importance of natural variation, paired with laboratory studies to provide a detailed understanding of the processes at work. The specific aims of our work are to (i) identify how the challenges of life in the wild affect infection and immunity (2) identify how parasites infecting the same host interact, and how those interactions affect host health, and (3) consider how parasites move between host species in a natural context.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>Our goal is to provide insight into what determines the consequences of parasite infection under real-world scenarios. Our results will improve our</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	current knowledge of how the immune system operates and prioritises its response to different parasites in wild populations, give us a better understanding of how parasites interact in variable ecological conditions and help identify priorities for medical intervention. Our findings will particularly be of interest to those involved in the design and outcomes of disease treatment programmes (e.g. deworming), given that there is considerable debate on the wider benefits of these programmes, but testing the potential benefits of alternative treatment strategies are difficult in humans, but feasible in wild rodents.
What species and approximate numbers of animals do you expect to use over what period of time?	To do this work, we will use up to 700 laboratory mice, 1200 wild mice and 1000 voles per year. In both the laboratory and wild, we will be careful to use as few animals as possible, while maximising the amount of information collected from each animal. Specifically we will use mice in the controlled laboratory setting to better understand how parasites and the immune response interact within a host. We will also use wild mice and voles to investigate how challenges of life in the wild affect the development of an immune response and the consequences of infection. We will combine these approaches with extensive statistical and mathematical modelling to ensure that we use the fewest animals possible, while broadening the implications of our research.
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?	It is crucial in our experiments that animals keep in good condition. The laboratory mice will experience infection with only mildly pathogenic parasites, which we do not expect to lead to more than transient discomfort and minimal weight loss. These mice will be humanely killed at the end of the experiment. For the studies of wild mice and voles, some animals will receive a drug treatment to treat or reduce their natural parasite infection. We expect that these drug treatments will most likely improve the health of the animal. Most animals will be released on site, however a subset of individuals will be humanely killed for further sampling.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because we are interested in how whole organisms deal with natural infections, our work needs to be performed in animals. However, wherever possible we adjust the numbers of animals needed to reach

	robust conclusions while minimising animal usage, replace with experiments that do not involve animals, and continually strive to improve our methods. Most of our work addresses phenomena that occur at the whole organ (e.g. spleen) or organism (e.g. effect of sex) levels, and up to the population/community levels (e.g. disease transmission across host communities). Part of this project, however, aims to use data generated in animals systems to build mathematical models that will help predict how the immune system/population will perform from much fewer samples or in other systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use controlled, replicated experiments to address our hypotheses, which provide far greater inference and, thus fewer animals, than purely observational surveys. In laboratory mice, lines are chosen to answer specific questions about the effects of variation (e.g coinfection). In our studies of wild rodents, we will take multiple sources of variation into account; for every animal at every trapping occasion, we record a range of individual and the environmental variables to ensure that we are able to control for potential confounding variables, that we are unable to control in our experimental designs. Through this we can minimise the number of animals needed in order to test the specific hypotheses of each experiment. Here, collaboration with quantitative ecologists and regular monitoring of the variability of recently collected samples help define an appropriate compromise between the appropriate number of animals needed to detect an effect while avoiding excess animal usage.
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.	For our laboratory work we use inbred laboratory mice as they provide a range of reagents not available in other species. In addition we will use a laboratory colony of wild-derived mice, as they are the primary natural hosts for the helminth and protozoan parasites we work with in the wild and have been used as models of immunity and coinfection. Wild mice and voles are natural hosts of a number of medically-relevant parasites. These species, and their parasites, have been studied for decades in

	the UK, and this knowledge base makes them excellent species to address our aims. Our live-trapping protocols are standard, and have been optimized to be as minimally invasive as possible. (e.g. with protective shelters, bedding and food provided). Most of the studies we conduct require good recapture rates and/or repeat sampling, and so it is essential the animals remain in good health. The stresses placed on individuals are minimised through good training and efficient field technique; our techniques have been established for >10 years across various projects and are optimised to minimise stress and reduce suffering.

Project 33	**Initiation, resolution and modulation of inflammation**	
Key Words (max. 5 words)	Autoimmunity; allergy; inflammation; T cells; therapy	
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)	Our objective is to understand the processes that control the unwanted and damaging inflammation that causes autoimmune and allergic diseases. Armed with this knowledge, we aim to develop novel approaches to the treatment of these diseases. Specifically, we will focus on the immune cells (T lymphocytes) that orchestrate the disease-causing inflammation. We already have a very effective means of switching off these unwanted T cell responses in our mouse models. To translate this into the most effective therapy for people with these diseases, we need to understand the mechanisms behind its effect. This might also lead to additional new drugs, some of which might treat only autoimmune disease, some which might only treat allergic disease, or perhaps some that can treat both of these types of 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)?	10-20% of the UK population suffer with autoimmune or allergic diseases. Currently, there are no cures and treatments often have serious side-effects. When we better understand the natural processes that the prevent the development of these inflammatory diseases (so-called “immune tolerance”), we can design more specific and more effective drugs.	
What species and approximate numbers of animals do you expect to use over what period of time?	We shall use mice throughout the project because they have the most fully characterized immune system. We estimate that we shall use 20,000 mice over the 5 years of the project. About half of this	

	number covers the breeding of genetically-modified mice required for the study.
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 the mice will experience mild to moderate effects. These include simple vaccination injections to provoke an immune response. In some experiments we shall subsequently need to provoke inflammation in the tissues. The models of inflammation in the skin and lung that we will use produce moderate disease. We shall also use models of multiple sclerosis and inflammatory bowel disease which produce more severe disease. However, we keep the use of this model to a minimum. All mice will be humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We study the development and resolution of immune responses. In particular, the induction of immune tolerance is a complex and dynamic process involving several populations of immune cells, which cannot be fully mimicked in the laboratory. However, we are currently attempting to develop meaningful in vitro assays that might be able to replicate some of the molecular changes that occur in immune cells during tolerance induction. If successful this will offer replacement as well as reduction benefits. It should be noted, however, that these in vitro assays still require immune cells isolated from genetically modified mice and that any information gained will ultimately need to be validated using some in vivo experimentation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed very advanced models that allow us to gain the most precise information on the function of disease-causing T cells. This means that the models are very reproducible and we have therefore been able to reduce the numbers of mice used, whilst still being able to gain statistically significant results.
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 have the most fully characterized immune system, with many tools available to study the function of T cells. In particular, we use T cells that have been genetically-modified so that they can induce autoimmune or allergic disease and/or are deficient in particular genes involved in the immune response. Also these T cells are traceable, allowing us to focus on those cells that are driving the inflammation. Using these mice allows us to gain most of the information we need from using models of

	inflammation that involve the least possible harm to the mice. Where necessary, mice receive appropriate anaesthesia during the procedure. In some experiments mice are at risk of infection. These mice are kept in clean cages and receive appropriate antibiotics. All mice are monitored regularly and any that show signs of ill health receive prompt veterinary intervention. If significant ill health is evident, the mice are humanely destroyed.

Project 34	**Type 2 immunity in infection and tissue homeostasis**	
Key Words (max. 5 words)	Helminth infection, cytokines, macrophages, wound repair, allergy	
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
		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 with multicellular parasites (helminths) leads to what is called a 'type 2' immune response. For reasons that are not fully understood, this arm of the immune system is involved not only in combatting infection with these large parasites but regulating wound repair and the body's energy balance. We strive to understand how the cells and proteins that are expanded during a type 2 immune response mediate these different functions.	
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)?	Helminths infect over a quarter of the human population and the vast majority of wild and domestic animals. Although rarely fatal, they affect mental and physical development in humans and can cause severe debilitating disease. The also represent an enormous economic burden to the livestock industry. Our findings will help develop strategies, such as vaccination, for controlling these diseases. In particular, we study parasites that in humans cause elephantiasis and river blindness. We also study parasites of the intestine that first migrate through the lung. These lung migrating parasites are very common in people living in developing countries. The type 2 immune response also is a major cause of allergy, asthma and tissue scarring (fibrosis). Fibrosis is a major cause of death worldwide but little is understood of its root causes. We expect our work to reveal important details of how these diseases	

	develop, helping in the future development of therapies. Diseases of metabolism are considered a major challenge for the western world and increasingly in developing countries. The unexpected discovery that cells and proteins of the 'type 2' immune response interact with fat cells to regulate metabolism will open the doors for novel approaches to treating obesity and diabetes. Most of the work we do is fundamental research designed to unravel the details of the interactions between cells of the immune system and the rest of the body. Therefore, we don't expect to generate or test therapies directly. However, the work we do will provide important knowledge for those involved in direct translational programmes.
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of animals we will use will be mice. We expect to use approx. 55,000 over 5 years. Approx. half of these will be used for breeding strains that have specific gene modifications needed to test particular hypothesis. We will also use approx. 5000 jirds and rats to maintain life cycles of the helminth parasites
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 animals will experience no adverse effects or only mild adverse affects. The parasite infections are generally well tolerated and will rarely even reach moderate severity. Some manipulations that make animals more susceptible to infection may increase the severity from mild to moderate. All animals will be killed before they exceed moderate severity limits.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our studies rely on looking at the immune response to infection or other conditions in the context of the whole body. We cannot replicate these processes outside the body. No alternatives for exist for parasite migration through the body, wound repair in the tissues, effect of dietary manipulation on parasite survival exist. Further, these processes involve multiple body systems. In particular we study how cells move through the body during disease, whether they expand at the site of infection and injury by dividing or whether they come in from the blood. These processes are tightly regulated by multiple systems in the body and cannot be studied in cell

	culture or artificial model systems. Whenever possible, we use cell culture systems to address specific questions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experiments are carefully designed such that the results will be clearly interpretable using the smallest number of mice. We consult statisticians whenever necessary to ensure this is the case. Additionally, we carefully discuss our experiments as a team to design experiments to take full advantage of all tissues in the animal. For example one person may be studying the body cavities while another studies the liver, and another the intestine, all in the context of a particular helminth infection. Thus, different tissues from one mouse can be used to answer multiple questions. This is a routine process in the lab. Additionally, through careful assessment of animal strains used, we have over the past 5 years dramatically reduced the numbers of animals needed for maintenance of parasite life cycles.
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 a variety of helminth models that infect different tissues of the body. When the natural host is a mouse, the mouse is used to keep parasites alive (none can be maintained in culture). In some cases, rats or jirds are more susceptible to infection and then these are used to maintain the parasite, so that we can use the fewest animals possible. We use inbred laboratory mice for the vast majority of our experimental work because of the enormous range of reagents that are available. These can be used to assess in detail the mechanisms by which the cells and proteins of the type 2 immune response cause wound repair, changes in metabolism and killing of parasites. For example, we use a parasite that migrates through the lung before it reaches the intestine. This model allows us to study mechanisms of worm killing in the lung and intestine, while at the same time studying how the lung is repaired, and how poor repair leads to long term lung problems. Animals are closely monitored for any ill affects, typically by visual assessment and weighing. Weight loss can predict ill effects before they are seen visually.

Project 35	**Does steroid production in inflammatory arthritis cause joint destruction, muscle wasting and bone loss?**	
Key Words (max. 5 words)	Rheumatoid arthritis Steroid metabolism Bone loss Muscle wasting Cartilage erosion	
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
		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 wish to know whether joint destruction, muscle wasting and bone loss observed in Rheumatoid arthritis (RA) are due to the effect of steroid production on the cell communication network known as 'Wnt signalling'. This will initially be studied in tissues from RA patients. Using animal models of arthritis that mimic what we see in RA, we will then address the 3 key questions. These are 1, how do steroids regulate 'Wnt signalling' in animal models of arthritis. 2, do genetically modified animals that lack this steroid production, offer protection against joint destruction, muscle wasting and bone loss. 3, whether specialised drugs, which block the production of these naturally occurring steroids, offer protection against joint destruction, muscle wasting and bone loss.	
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 study will help us better understand the mechanism behind the harmful consequences of RA, creating new opportunities to develop treatments, which target steroid production and ‘Wnt signalling’. Using mouse models of arthritis, this study will also determine whether currently available drugs that block steroid production, have therapeutic benefits in the treatment of inflammatory disease. If successful,	

	this would pave the way to test these drugs in people with RA in the immediate future. Ultimately, this could lead to improvements in patient quality of life, reducing disability and the associated costs to the NHS and wider economy.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. This study intends to examine approximately 950 animals 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?	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 be killed to prevent any on-going discomfort. Animals presenting with moderate symptoms of pain or discomfort will receive analgesics to manage this. 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 be monitored closely throughout procedures and killed where adverse effects are identified to prevent any on-going discomfort.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-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 blocking the production of steroids will protect against joint destruction, muscle wasting and bone loss. Essentially, this will provide the validation and rationale to examine this in human inflammatory disease such as RA.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Carefully refined statistical analysis will ensure that we use the least number of animals to provide a meaningful answer to our research questions. Throughout the project our work is supplemented by studies in cells that reduce dependence on animal. Another 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. Importantly, in pilot studies informing this project we have discovered that the therapeutic drug Remicade, used in the clinical setting to treat RA, is effective in treating disease symptom in our animal models of arthritis. When used in our breeding programmes, we now require significantly fewer animals to successfully generate our experimental animals. Pilot studies throughout using small experimental numbers will ensure that no large experiments will go ahead unless they will provide meaningful results.
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 best model for the study of persistent disease because: (i) 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 (ii) A wide range of wild type and genetically manipulated strains of defined genetic makeup are available; (iii) An extensive range of reagents is available for analysis of immune responses (iv) 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. (v) There are no other alternatives to this work. A major refinement undertaken for this project includes the administration of the of therapeutic drug Remicade, used in the clinical setting to treat RA. This significantly improves disease symptoms, quality of life and lifespan in animal models of arthritis required to breed experimental animals.

Project 36	**Polarised secretion from lymphocytes**	
Key Words (max. 5 words)	Immune cells, cancer, viral infections.	
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 certain white blood cells, called “killer cells” are able to defend the body against viral infections and cancer. Killer cells are very efficient cells of the immune system, that patrol the body seeking out virally infected and cancerous cells. When they find a target they can kill the infected or cancerous cell rapidly and precisely, without destroying neighbouring healthy cells. At the moment, relatively little is known about the mechanisms that controls the killer cells and so we do not know how to help them kill better when cancer begins to overwhelm the immune system. In addition we sometimes need to dampen the effects of the killer cells as, if they recognise healthy cells they can cause autoimmune conditions such as type-I diabetes.	
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 we could discover the mechanisms controlling the functions of killer cells then we could help find ways to modulate their activity. We would have clues that would help us find drugs that would improve treatments for cancer and for autoimmune conditions.	
What species and approximate numbers of	We will use either mice or rats and over the next 5 years; we have estimated that we require 2730 mice	

animals do you expect to use over what period of time?	and 100 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?	Mice with specific modifications of interest in their genetic make up will be bred and killed humanely so that their tissues can be used to isolate and study killer cells of the immune system. The genetic modifications are not expected to give rise to any harmful effects. We will immunise some of the mice in order to generate antibodies that can be used to study proteins involved in secretion. Therefore some mice will receive 'immunisations', similar to the process used in childhood vaccinations. Occasionally there may be some soreness after immunisation, but this should resolve quickly. If soreness does not resolve readily, the animal will be humanely killed in order to avoid any suffering. At the end of all experiments animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Genetically engineered mice allow us to grow killer cells and study the inner machinery that makes them work. Mice and rats can be used to make antibodies that can be used to identify important components of the machinery within killer cells, and can also produce a new and successful type of medicine known as a 'monoclonal antibody'.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We constantly refine our techniques for growing killer cells in the lab, so that we can reduce the number of animals that are required. Using genetically modified mice allows us to use ten fold fewer animals than we would otherwise use as the genetically modified mice can produce more killer cells per animal.
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.	Regular blood sample checks will be used to establish when a good response is achieved in order to minimise the number of immunisations required. The use of genetically altered mice will allow us to generate very large numbers (>10^9) of killer cells from each animal. All animals are monitored for any signs of ill health and discomfort. Adverse effects (eg granulomas) are extremely rare in this project, but if they do occur and cannot be treated by minor intervention the animals will be killed humanely.

Project 37	**Neonatal bacterial meningitis – infection and treatment**	
Key Words (max. 5 words)	*In vivo* imaging; *Escherichia coli* K1; blood-brain barrier; Group B streptococcus; neonatal bacterial meningitis	
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)	Neonatal bacterial meningitis (NBM) is a disease affecting newborn babies in both industrialised and developing countries. With a mortality rate of 15-50% globally, even for survivors the consequences can be seriously debilitating. NBM is a complex disease which involves several steps of pathogenesis. As bacteria are normally taken up by the infant orally during birth, they must then colonise the gut and transition into the bloodstream where they replicate and may cause sepsis and/or migrate into the brain. We propose to use murine models of NBM to define the bacteria-host interactions that enable bacteria to breach the host's barriers. To do this we will set up *in vivo* imaging models where the infectious organism is genetically modified to express proteins that will glow when the organism is imaged. Following infection, these glowing bacteria can then be tracked using whole animal non-invasive imaging. As the disease progresses the location and concentration of infection can be determined at multiple timepoints in the same animal. Importantly, how different treatments may alter disease progression can then be visualised in different experimental groups. We wish specifically to look at how the components of the host cell membrane modulate bacterial invasion of host cells, which in turn affects the way in which bacterial infection will progress. To alter these components we will use genetically modified or	

	immunosuppressed mice, or we will test agents that will modify membrane constitution, or through a fatty diet. This might affect gut and brain barrier cells as well as macrophages in the blood, and we will look for effects at the different stages.
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)?	Based on preliminary experiments in cell culture and in the mouse neonate model, altering cholesterol levels in the host cells has the potential to prevent bacterial invasion into the brain. This would restrict bacteria to the blood and would make them more accessible for antibiotic treatment and should lead to a reduction in the severity of brain damage. Identification of therapeutic agents for clinical studies would have the potential to prevent the damaging neurological sequelae in surviving human infants as well as to decrease mortality rates.
What species and approximate numbers of animals do you expect to use over what period of time?	We would anticipate using both mouse and rat neonates, approximately 2000 of each over the course of the project time 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 anticipate that the animals may feel unwell to the extent that they might experience a change in body temperature, weight loss of up to 10% or changing feeding patterns. Most animals will be humanely culled before they become severely ill (i.e. lose more than 10% of body weight or stop feeding for more than a day) at the end of any experiment to enable collection of blood and tissue samples to measure bacterial load. In order to fully recapitulate the human disease progression, a small number of animals will be allowed to feel very unwell, i.e. they may lose more than 10% of body weight or become less mobile, and they will be humanely culled before they become moribund.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	NBM has been studied using both *in vitro* and animal models. Cell culture models can only simulate specific stages of the infection process, and in order to mimic the human disease as closely as possible, to dissect the different stages of disease pathogenesis and to investigate the effects of potential treatments on disease outcome, it is necessary to look at the physiology of the whole organism during infection.
2. Reduction Explain how you will assure	Infection experiments usually require one to two litters of rat pups for one treatment, two to three litters

the use of minimum numbers of animals	per treatment for the mouse model. We have determined that in short-term experiments, there is no cross-infection within a litter, and therefore by assigning random treatment to individual littermates we can decrease sample size. Furthermore use of bioluminescence imaging enables collection of data from the same animal through the experiment, rather than culling animals at each timepoint.
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.	Rat and mouse models of NBM associated with *E. coli* K1 infection have been described. The mouse model uses an inbred strain, which enables the benefit of genetically modified animals to address questions on the host-pathogen relationship. The rat model is long established in the preclinical field, however it uses outbred rats, and we would wish to investigate inbred animal strains to allow the use of smaller groups of animals. As it is hard to distinguish symptoms of illness in neonates such as ruffled hair used in adult animals, we have employed a clinical scoring system to determine endpoints that minimise animal suffering. Whole animal imaging is noninvasive and highly sensitive, and may detect infection in an animal before comparable symptoms are apparent. This imaging approach may predict disease progression earlier than using conventional visible symptoms and lead to refinement of experimental endpoints.

Project 38	**Immune responses promoting inflammatory disorders**	
Key Words (max. 5 words)	Innate immunity, cancer , inflammation, imaging	
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
	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 is a process initiated by infection and tissue injury. A tightly regulated equilibrium between innate and adaptive immune cell responses allows an efficient response to take place and to properly resolve in damaged tissue. The deregulation of this complex network is known to lead to pathological inflammation including cancer. Yet, the cellular and molecular regulatory pathways involved in these pathologies are poorly understood and the series of events occurring *in situ* during pro-inflammatory responses remain undiscovered. The proposed studies will investigate the complex immune network established upon chronic inflammation and tissue repair that favour cancer progression. It will also address the real time location and migration of innate immune cells in inflamed tissues. As the project progresses, implementation of in silico computer modelling studies will be pursued in order to identify particular molecular pathways and predict specific experiments, replacing a significant number of animal-based experiments with computer stimulations.	
What are the potential benefits likely to derive from this project (how science could be	Cancer associated with chronic inflammation represents a type of cancer that could be prevented by tailored, anti-inflammatory treatments. This project	

advanced or humans or animals could benefit from the project)?	will generate knowledge that can help design new therapeutic strategies against cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse/ 3000 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?	Overall level of severity is Moderate Genotoxic agents, some immunostimulant and irradiation associated with hematopoietic reconstitution can perturb haematopoiesis homeostasis. The use of infectious agent is likely to damage the epithelial barrier integrity and may lead to weight loss, lack of activity, hunched posture and diarrhea in the case of gut tropism. In investigations of cancer models, expected adverse effect generally includes distress caused by tumour development. Finally, besides risks of infection associated with surgery, adverse effects could arise from unchecked variations in the level of general anaesthesia. Animals will be checked daily during any post-operative phase and daily during any period of cell/substance administration and upon cell transplant post-irradiation to ensure proper hematopoietic reconstitution. Increased frequency of monitoring may be undertaken for certain procedures and remedial action taken as advised by the NVS. Aseptic technique will be used to avoid infection as a consequence of surgery. Where necessary, animals will be provided with softened food to limit the effects of certain symptoms on feeding. Mice will be sacrificed by schedule 1 method at the end of the studies. Experiments will be immediately terminated if signs of anaesthetic complications/ physiological distress are observed during surgical procedure. Animals will be immediately killed should post-surgical complications arise or if animals reach humane endpoints established in addition to experimental endpoint in cancer studies.
Application of the 3Rs	
1. Replacement State why you need to use	Several questions raised by this proposal rely on the use of animal models since *in vitro* studies have

animals and why you cannot use non-animal alternatives	many shortcomings. Some *in vivo* studies such as systemic infection, tumor formation or autoimmune disorders have no *in vitro* counterpart. Even in well-characterized *in vitro* systems, it has proven impossible to accurately mimic the *in vivo* environment, which involves a complex lymphoid architecture, dynamic of the immune system and interactions of the cells. In addition, part of the study proposes to investigate the dynamic of the immune response during tumour progression. Our strategy will be to use intra-vital imaging for that purpose which can only be done in vivo, using animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Colonies will be carefully monitored so that excessive breeding of animals is avoided. Experimental design and statistical analyses have been performed to generate the minimum number of mice needed to reach substantiated conclusions. As part of good laboratory practice and to limit unnecessary repeated experiments, clear and detailed recording of animal health status is kept by laboratory researchers, which will reviewed by the project leader.
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 these experiments because of the availability of inbred and genetically altered mice. The fast breeding time, the availability of well-developed procedures and reagents, and the accumulated body of information on murine immune system justifies the choice. Also, the mouse and human immune systems are very similar in their properties. Finally, a phenomenon that is unlikely to be discovered in humans, because direct experimentation is not possible, can often be discovered in mice and generalized to humans. This research program does not involve experiments of substantial severity; the maximum level of severity is moderate. Each regulated procedure involves regular and careful monitoring of the colonies using a well-established scoring system for disease progression; general anesthesia is used to relieve pain from surgery, and antibiotics are used to prevent infection as directed by veterinary surgeons. Humane end-point has been established for each adverse effect.

Project 39	**Production of *Hymenolepis diminuta* ova in rats**	
Key Words (max. 5 words)	Hymenolepis diminuta ova rat tapeworm	
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 objective of the project is to provide researchers with a source of rat tapeworm eggs which will allow researchers to obtain tapeworm larvae. The larvae will then be used to develop improved methods for their isolation, cleaning and storage and this will be of benefit to researchers who have an interest in the use or study of the tapeworm larvae and their significance in humans with allergies and other problems with their immune system.	
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 aim of this project licence is to provide rat tapeworm eggs to researchers who will use them to be able to establish a ready supply of the larval form of the tapeworm for their research. The eggs will allow us to establish a reliable source of larvae and this in turn will allow the conduct of research into efforts aimed at extending our ability to store them for longer periods. The larvae are also required for study with regards to their potential benefit for treatments of autoimmune and allergic conditions in Humans. There is currently no commercial source of eggs or larvae of this tapeworm in the UK or Europe.	
What species and approximate numbers of animals do you expect to use	Rats. Up to 50 rats over a 5 year period.	

over what period of time?	
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 tapeworm we are working with (*Hymenolepis diminuta*) naturally colonises the common rat population throughout the word. The mature tapeworm lives in the small intestine of the rat without clinically apparent adverse effects on the rat, and it produces eggs which are passed in the rat faeces. As part of this licence, to establish the tapeworm in rats, tapeworm larvae will be fed to laboratory rats in a small amount of fluid or on their solid feed.If the tapeworm eggs are not subsequently found in the faeces, a second dose of larvae can be fed to the rats. This need only occur once or twice during the rat's life span to ensure the rats remain infected with the tapeworm.The expected level of severity of this project is 'mild' and animals may show no observable adverse effects. The rats will be housed for the full period of their natural lifespan of 1.5 – 3 years and they will die a natural death unless they develop illness which causes pain or discomfort in which case they will be humanely killed. Rats remaining alive at the end of the project will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The rat tapeworm *Hymenolepis diminuta* - can only survive and reproduce inside the rat - its natural host. As yet, there are no non-animal alternatives for the production of tapeworm eggs which are needed to produce tapeworm larvae.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project will start with 1 pair of rats. Extra rats will only be used if there is sufficient demand for tapeworm eggs to justify an increase. Should demand for the eggs diminish, the number being maintained can be reduced by humane killing of the rats.
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	Rats are the research model of choice because the tapeworm (***Hymenolepis diminuta)*** is a natural inhabitant of the intestines of wild rats. The tapeworm is expected to cause no discernible detrimental effect on the rats. Therefore the rats will not be subjected to any stress or stressful procedures, other than that associated with the routine animal husbandry tasks involved with keeping the rats in the laboratory

minimise welfare costs (harms) to the animals.	environment for their lifetime. Rats will be housed in cages which will meet the legally required sizes and the cages will contain objects and materials for enriching the environment of the rats. Most importantly, the rats will be housed in pairs, giving the rats companionship. The rats will be monitored and cared for by competent animal technicians. The Named Veterinary Surgeon and Named Animal Care and Welfare Officer will be involved in recommending and providing appropriate husbandry and veterinary care.

Project 40	**Drug assessment in models of multiple sclerosis**	
Key Words (max. 5 words)	Multiple sclerosis, therapy, immunology, demyelination, remyelination	
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 objective is to provide a suite of advanced, mode-of-action models that will allow the pharmaceutical and biotechnology industries to test novel and existing drug candidates for use in multiple sclerosis (MS). In vivo models are combined with advanced in vitro assays that allow us to study the effects of the test drugs either on inflammation driven by the immune system within the brain, or on regenerative processes within the brain that restore nerve 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)?	The direct outcomes of this project will be data that can inform the drug development programmes of Aquila BioMedical's clients. This allows those clients to decide on which candidate drugs are most likely to prove beneficial, ultimately in patients. Approximately 100,000 people in the UK suffer with MS. There is no cure and, whilst some current treatments can be very effective, not all patients respond and the drugs can have serious side-effects. There is still a need for better, safer and more specific drugs. In particular, there is no current treatment option for patients with advanced MS, in who the nerve damage cannot currently be reversed. The identification of drugs that could trigger such recovery of nerve function would be a major advance.	

What species and approximate numbers of animals do you expect to use over what period of time?	We shall predominantly use mice, but some rats may also be used, if the drugs being tested are known to be more effective in rats than mice. Over the 5 years of this project, we anticipate using up to 10,000 animals. This number includes the breeding of 3,000 genetically-modified 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 majority of the genetically-modified mice will be humanely killed to provide cells and tissues for use in the laboratory and will undergo no further experimentation. Beyond our breeding program, the majority of the animals used will experience mild to moderate effects. These include simple vaccination injections to provoke an immune response. In some experiments we shall subsequently need to provoke inflammation in the brain to mimic MS. This can lead to severe disease (paralysis, which normally resolves). A small number (around 50) of the genetically-modified mice might spontaneously develop signs of this disease. When this is identified, those mice are humanely killed immediately. We shall also use models in which chemicals are administered which temporarily affect nerve function in the brain. Usually this produces a less severe form of impairment. All animals will be humanely killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The processes that lead to MS are not fully understood, but include an unwanted immune response in the brain, leading subsequently to nerve damage that cannot be repaired. These complex interactions cannot be fully replicated by standard assay in the laboratory. That said, we make extensive use of lab assays to provide screening platforms to identify candidate drugs that are most likely to succeed in the animal models of disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have developed very advanced models that allow us to gain the most precise information on the function of disease-causing immune cells. This means that the models are very reproducible and we have therefore been able to reduce the numbers of animals used, whilst still being able to gain statistically significant results. Our lab screening platforms also mean that we can identify the drugs that are most likely to have beneficial effects in vivo. Focusing on these drugs allows us to further reduce the numbers of animals used in disease 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.	We mostly use mice because they have the most fully characterized immune system, with many tools available to study the function of immune cells. In particular, we use T cells that have been genetically-modified so that they can cause MS-like disease. Also, these T cells are traceable, allowing us to focus on those cells that are driving the inflammation. Using these mice allows us to gain most of the information we need on how their activity has been altered by the drug being tested. We will also make use of screening models in which these T cells drive inflammation in the skin, rather than the brain, thereby causing least possible harm to the animals. Some experiments will need to use the equivalent disease models in rats, because some drugs are known to work specifically in rats rather than mice. We have well-defined scoring systems and monitoring regimes with strict humane endpoints. Where necessary, animals will receive appropriate anaesthesia during the procedure and analgesia following surgery. In some experiments animals are at risk of infection. These animals will are kept in a barrier environment and receive appropriate antibiotics. All animals are monitored regularly and any that show signs of ill health receive prompt veterinary intervention. If significant ill health is evident, the animals are humanely destroyed.

Project 41	**Enhancing the value of selective breeding for parasite resistance**	
Key Words (max. 5 words)	Parasitology, livestock, genetics, immunology, pathology	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		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 aim is to improve roundworm parasite control in British sheep by selective breeding. The objectives are to improve our understanding of the infection process and use this improved understanding to develop better markers to identify resistant animals and to inform mathematical models of the infection process to better predict the response to selection.	
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 infection process will lead to better identification of resistant livestock and faster responses to selection. Better mathematical models will predict the benefits of selective breeding more accurately. More use of resistant livestock will improve disease control with benefits for animal welfare and the sustainability of livestock farming.	
What species and approximate numbers of animals do you expect to use over what period of time?	5250 sheep and 1250 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?	Most animals will only experience mild discomfort from providing a blood sample. A small number of animals will be deliberately infected with nematodes and this may cause moderate discomfort similar to that experienced by most grazing animals.	

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no disease model for nematode infection that can be used to study genetic variation, immunological responses or the development of disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Advanced statistical methods will be used to determine the appropriate sample size for each 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.	Sheep are the target species. Each species responds differently to infection and model organisms do not predict the protective immune response. Blood, saliva and faecal sampling are the least invasive procedures that can provide the necessary samples for genetic, immunological and parasitological analysis. We are moving from blood to saliva as a source of antibody which will minimise distress. In order to study immune responses to nematodes we need a source of parasite material. Previously, this required deliberate infection. However, we have recently cloned, sequenced and expressed a recombinant antigen (tropomyosin). Responses to tropomyosin are associated with protection. We will continue to study the immune response and use recombinant targets wherever possible. The use of recombinant proteins to study immune responses in vitro reduces the need to deliberately infect sheep to obtain parasite material. We have also constructed a mathematical model that can minimise the number and size of experiments that need to be performed.

Project 42	**Pathogenesis mechanisms of bacterial pathogens**	
Key Words (max. 5 words)	Macrophages, innate immunity, *Listeria*, *Streptococci*, *Staphylococci*	
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)	Infections caused by bacteria are a major health burden worldwide and a threat to society and health systems. New bacterial strains that can cause disease in humans and animals are constantly emerging. Current treatment options are in danger of becoming ineffective as multi-drug resistant bacterial strains emerge. New approaches for prevention and treatment of bacterial infections are thus urgently needed. This project investigates how cells of the immune system called macrophages recognise and destroy bacteria that invade the body. Macrophages are key cells of host defence that orchestrate a coordinated immune response to any infectious agent. Macrophages recognise and eat bacteria, which triggers inflammation and immune responses. Many of the mechanisms of how they do this are still unknown. We will use mouse models of infection to study the function of genes and molecules that play a role in macrophage host defence. We will also look at mechanisms of how disease-causing bacteria can evade macrophage defence reactions. In our mouse models we will study three major types of bacteria that can cause severe disease in animals and humans. We will use mouse models of	

	Listeria monocytogenes infection to study a food-borne disease called listeriosis. It can cause sepsis, severe brain infections, and abortions in susceptible patients and pregnant women, respectively. We will also investigate the interaction of another bacteria called *Streptococcus pyogenes* with macrophages and study how it can induce cell death to evade immune responses. *Streptococci* are also major cause of sepsis in humans when they manage to reach the blood stream. Finally, we are studying how *Staphylococci* bacteria colonise the host and invade the skin to cause abscesses and skin infection. Here we have established a mouse model of canine skin infection to identify vaccines against the pathogen. Using a chicken model of *Staphylococcus aureus* infection, we are investigating how these bacteria that have originally come from humans, have adapted to avian hosts to cause disease. We are studying how these bacteria can infect the bone of chickens. The resulting inflammation called chondronecrosis is a major cause of lameness and a significant welfare issue in the broiler chicken industry.
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 is designed to investigate the basic mechanisms of how macrophages recognise and defend against bacteria. We envisage that our research will discover new receptors and signalling molecules that macrophages use to defend against infectious agents and to alert other cells of the immune system to the incoming danger. These new molecules may be useful as new drug targets for improving host defence or to alleviate excessive inflammatory reactions that are associated with severe infectious disease (e.g. sepsis, abscesses, bone infection). Other key benefits of our project are the potential development of new vaccination strategies that will target macrophages as important cells for activating the immune response. Our study of factors that make the bacteria more powerful might lead to new drug and vaccine candidates that can be used to protect against infectious disease. We will also try to improve our understanding of how bacteria adapt to new hosts. We hope to discover mechanisms how *Staphylococci* bacteria can infect chickens. Such knowledge can potentially be used to improve the welfare of chickens in the poultry industry.

What species and approximate numbers of animals do you expect to use over what period of time?	Mouse (wildtype and genetically modified). Up to 12400 mice over 5 years. Chicken (wildtype and genetically modified) up to 800 chickens 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?	Potential adverse effects may include the appearance of clinical signs of disease after infection, such as local or systemic infections and tissue inflammation. To minimise adverse effects we are monitoring infected animals closely using standardized protocols. This is important as if we did not monitor them carefully, some of these infections could potentially lead to death. However, we will interfere before this happens and will kill the animals humanely.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex host response to an invading pathogen involves many different tissue and immune cells. Macrophages are very heterogeneous cells that carry out different functions depending on the tissue or organ they are residing in. Currently, we don't have tissue cultures systems for all different types of macrophages available that would allow us to study interactions of the cells with pathogens and other immune cells. Whenever possible we will use cell culture systems and cell lines to analyse particular host defence reactions that don't need a whole animal system. We have recently developed new approaches that allow manipulation of host defence genes in macrophage cultures. These systems will replace and supplement *in vivo* animal experiments to a significant extend. Our aim is to develop these technologies further over the course of this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Group sizes of experimental animals are calculated to keep animal numbers to a minimum, whilst ensuring that numbers are used that can provide statistically significant data. Required animal numbers are constantly reviewed based on experience, previous data and statistical advice. Macrophage cell lines and macrophages grown *ex vivo* from the bone marrow will be used to replace *in vivo* infection challenge experiments. As mentioned above we have developed new methods to ablate genes of interest in cultured macrophages which will allow us to study particular aspects of their functions *in vitro*.
3.Refinement Explain the choice of species	We have chosen to use mice in our studies because the mouse as model systems provides us genetic tools and reagents that we need to dissect functions of

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.	immune cells and host defence genes at a whole animal level. The mouse is unique in this regard as a mammalian model system. Chickens are used as an experimental system because the *Staphylococci* bacteria that we study are naturally adapted to avian hosts and there is currently no alternative system available to answer our research questions. Animal suffering is minimised through close and standardised monitoring of health status during infection using a well-established scoring system. Animals are euthanized when defined human endpoints are reached or when pre-defined clinical endpoints are reached during an infection cause.

Project 43	**The Production of Antibodies**	
Key Words (max. 5 words)	Antibody, Polyclonal, Monoclonal, Immunogen, Antigen	
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 objective of this licence is to provide a service for the production of antibodies for both the medical research community and diagnostics manufacturing industry within the UK and Europe. Antibodies are produced by the immune system of a living organism and play an integral role in Biology in terms of their ability to fight infection by a host of organisms deemed foreign to self, their ability to detect life threatening disease and their use as critical tools in the areas of research and medicine, including basic research of cells and their function in disease, diagnostic technology and therapeutic medicine development.	
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)?	Antibodies routinely help scientists to research the function of both healthy and abnormal cells in disease, by the detection of proteins within the cell at various stages of its development. This is routinely utilised when researching disease and its prevention. In the field of diagnostics antibodies play a critical role in the detection of disease in a clinical environment. This can allow for the rapid diagnosis of life threatening disease and assist in providing clinicians (clinical Scientists and Doctors) with specific information in terms of the most appropriate course of treatment to follow, thus preventing death. The use of antibodies in therapeutics is a fast developing area of medicine, with the use of antibodies as constituents of direct medicine in order to treat various diseases including cancer, auto-immune	

	disorders and infection.
What species and approximate numbers of animals do you expect to use over what period of time?	Rat 3,500 (5 Years) Mouse 6,500 (5 Years) Guinea Pig 2,500 (5 Years) Rabbit 12,500 (5 Years) Chicken 750 (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 production of custom antibodies requires the use of live animals, to which a substance, called an antigen, is introduced to produce an immune response. To assist in the development of an immune response to an antigen a substance known as an adjuvant can be used in conjunction with the antigen to assist in the further stimulation of the immune system and subsequent production of antibodies. Some adjuvants which are very effective in stimulating an immune response can cause tissue reactions in the animals at the site of injection, therefore the use of these adjuvants is carefully controlled, with any reaction being closely monitored. Subsequent blood samples will be taken from an animal in order to test the level of antibodies being produced within the animal. These blood samples will be taken from an appropriate collection site on the animal such as veins/arteries and as such can (but rarely) lead to the formation of bruising and slight skin damage. When raising antibodies against DNA, special technology has been developed to do so. This technology involves the use of DNA coated gold particles which are introduced to the animal via bombardment of the skin with pressurised gas. This procedure is carried out under general anaesthesia and has minimal associated effects, which can include slight redness of the skin at the site of inoculation. The production of antibodies against bacteria requires the inoculation of a bacterial liquid(without adjuvant) direct into the blood stream. This methodology can result in the loss of animal body weight and (rarely) the onset of symptoms that appear similar to that of an allergic reaction e.g. laboured breathing, reduced mobility and redness of the eyes with associated light sensitivity. Upon reaching a desired level of circulating antibody to an antigen an animal will be moved forward for exsanguination where animals are given an anaesthetic from which they are not allowed to recover and their blood is collected to provide the antibodies. When this has been done the animal is humanely killed and further tissues may be collected for scientific use. Although significant adverse

	signs within any animal used for the production of antibodies are not expected full veterinary attention will be provided should there be any unexpected consequences of any procedure carried out. All animals used for the production of antibodies under the authority of this licence are subject to well defined humane endpoints, which if experienced will result in the animal being removed immediately from the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At the time of writing this licence there are no alternative (non-animal) methods for the production of blood serum containing a wide variety of antibodies to various targets or the production of specific (monoclonal) antibody secreting cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of appropriate species and methodology will ensure the production of better quality and a higher number of antibodies, therefore reducing the number of repeat production programs required where use of additional animals would be needed. Our expertise and experience in this area allows us to provide guidance on best practice from the beginning, this includes the selection of appropriate species based on the substance to which the antibodies are to be raised against, the way in which the substance will be introduced to the host and the schedule of inoculations to be followed. With all of these considerations we can ensure that the minimum number of animals are used for each and every project undertaking.
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 variety of species (Rabbit, Guinea Pig, Rat, Mouse & Chicken) will be made available for the production of antibodies. Selection of a specific species , from one of the above will be made following the careful consideration of a number of factors, both ethical and scientific. Our experience in this field allows us to make ethically sound decisions based on knowledge and expertise as well as ensuring the highest levels of care and attention are afforded to all animals utilised in the production of antibodies. Our production protocols are designed with the principles of minimal severity and are under constant review to ensure best practice is followed at all times, whilst also keeping abreast of new and refined techniques/technology utilised in the field of antibody production.

Project 44	**Mechanisms of lymphocyte activation**	
Key Words (max. 5 words)	Lymphocyte, immunoresponse	
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)	Infection Is one the greatest cause of premature mortality throughout the world, only surpassed by ischemic diseases, Vaccination is extremely important in reducing or eliminating certain infections, but there is clearly still progress to be made in their design and in optimising vaccine strategy. Our studies focus on specialised white blood immune cells that naturally protect us against invading pathogens by, for example, producing antibodies. We study the mechanisms that activate these cells and investigate how they interact with each other and with pathogens. We address these questions at the molecular and cellular levels using cutting-edge technology and under physiological conditions.	
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)?	We anticipate that the information gathered from work carried out under this Project Licence will make a valuable contribution in terms of advancing our general understanding of the immune system. Our studies on immunisation and infection should help us to unpick the molecular and cellular mechanisms that occur during the course of infectious diseases and could be important in terms of the design of effective and long-lasting vaccination strategies. Furthermore, given the enormous therapeutic value of monoclonal antibodies in the treatment of cancers and autoimmune diseases, we expect our studies to prove useful in the design of	

	improved antibody therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	We will produce up to 50,000 genetically altered animals over the 5-year period of the licence, 30% of which will be used in experiments. The remainder will be the animals necessary to breed those used in experiments. Wastage minimised by careful planning of breeding, and, where possible, littermates with the relevant genotype are used as controls, such as cre-negative or non-genetically modified from hetero/hetero crosses.
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 are careful in all our experiments to ensure that we use procedures that cause the least possible suffering. Some of our experiments call for the depletion or replacement of certain groups of cells with cells from source mice. For bone marrow transplantation recipient mice will first be treated with low doses of ionizing radiation before reconstitution of the bone marrow. In less than 5% of these mice, signs of engraftment failure such as persistent lethargy or weight loss may occur, in which case they will be humanely euthanised. In studies where infection is required, we use commonplace, well-known infectious agents such as influenza, and at very low doses, so the mice will experience similar side effects to those undergone by anyone immunised with childhood diseases or 'flu, and they recover just as quickly. These side effects are transient, and undetectable for 90% of the time, and the mice develop a normal immune response with subsequent recovery. Extremely occasionally, and only if there is a special scientific interest, a mouse experiencing a moderate reaction may be allowed up to 48 hours to develop a response leading to recovery. Some of the mice undergo imaging studies, and in these, less than 10% will carry an implanted window to allow the possibility of repeated imaging. We expect mice to make a reasonably rapid recovery from this procedure. Overall, we anticipate that many of the animals used in our experiments will experience undetectable or mild side effects. In actuality no more than 20% should experience even moderate severity. Once we have obtained the information we need from the experiment, mice will be euthanized humanely.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This is an area of great therapeutic potential with new antibody based-drugs for cancer licensed in the last year. However, the complex interactions cannot be replicated in any culture systems, meaning that animal experiments are required. Nevertheless, our group possesses an excellent current awareness of the scientific literature, specially with regard to immunology, making good use of wide-ranging literature searches and tools such as the 3RS' protocol design resources https:/lwww.nc3rs.org.uk/experimental- design) to both refine our research questions and to determine the relevance of the proposed animal models. We consult colleagues having complimentary research interests to avoid duplication of results (including negative results). We also make best use of preliminary experiments using or conventional cell culture methodologies or, where at all possible, replacement in vitro experiments using human blood products and cell lines. This means that before we consider using mice we have already explored our hypotheses in great detail. However, despite this, we cannot completely mimic the highly specialised tissues in which lymphocytes are activated and produce antibody using only these in vitro techniques. It is therefore necessary to perform experiments with mice. In addition, our aims of studying how lymphocytes communicate with other immune cells and how this may affect antibody production need to include in vivo analyses of the immune system and visualisation of where and when lymphocyte-antigen and cell-cell interactions take place.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The use of replacement systems for many of our experiments means that we have well formulated hypotheses before we begin mouse studies. This means we have realistic ideas about what to expect and can perform statistical 'power' calculations to work out how many mice are required to give a clear answer to the question we are asking. Where possible, we obtain mice that already have the genetic alterations needed for our experiments, which means that many fewer mice are needed for breeding. In addition, by using genetic systems that alter only certain types of immune cell we obtain our answers faster, again using fewer mice.

	We also try to maximise the information we get from each mouse. With good planning, we ensure that the blood and other tissues from any one mouse are fully used, often in more than one experiment, so we further increase the amount of useful information. Also, by using imaging to measure immune tissues in the same animal on more than one occasion we get a much clearer picture of how an infection proceeds than by comparing the stages in different mice on different days. We have an on-going goal of increasing our expertise in high-resolution cuffing-edge microscopy. In conventional light microscopy the resolution is limited, so that fine details are blurred in the image. The use of superresolution microscopy allows us to obtain the best possible images resolving fine details which might increase differences in our studies The resultant larger effect size gives the potential for us to achieve statistical significance with fewer experiments. We ensure our data are reported fully and promptly so that other researchers can benefit from our findings as soon as possible, which helps avoid repetition of 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.	Our group is actively engaged in trying to refine our protocols and procedures to minimise adverse effects. We have included a new protocol in this licence to be used wherever possible, where only the cells and tissues from the animals are used for experiments. This means that apart from their genetic background, they will not undergo any experimental procedures in their lifetime. We aim to address the needs of the mice and are always aware of the animals' welfare, being assiduous in updating and refining our husbandry according to current best practice. We work closely with the animal units to ensure the mice are kept in good conditions. All mice are kept in the most up-to-date facilities available, with individually ventilated cages, access to light according to natural light/dark patterns, and to continuously available food and water. They have daily visual checks by trained staff, and environmental enrichment such as sizzle nest bedding and mouse houses to encourage and facilitate their natural behaviours. When mice undergo experimental procedures, we consider the potential effects on the animals' welfare and take appropriate steps, including

	extra checks, to avoid or minimise discomfort/distress. For all our experiments we work with well-known systems and we ensure that we design our studies to elicit the fewest and least severe adverse effects possible. E.g. we employ cutting-edge genetic techniques to modify immune molecules in only the cell types of interest, reducing the impact on the whole animal. We try to use litter-mates as controls (with same genetic background but not carrying mutations) and where possible we also use mice as their own control or for duplicates to reduce the numbers. However, we are always aware of possible conflicts between reduction and refinement and if we felt we would otherwise be likely to increase the severity for the individual we would use separate mice. To understand how immune cells are activated and interact in our bodies it is important to use animals with fairly similar bodies, such as mammals. Much of our work involves understanding B cell activation and infection. This means that our studies require animals with similarly complex lymphoid tissues to humans. Mice are the most practical mammals to use that enable us to answer those scientific questions that require animal use (based on ease of husbandry, breeding rates, relative ease of genetically manipulation, and an extensive existing knowledge base).

<table>
<tr><td>Project 45</td><td>The immune system as gateway for mental and physical wellbeing</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Emotion, Immune-response, translational medicine</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Do you remember feeling very “low and sad” the last time you had a cold? Or the time you were really stressed and kept coming down with flu or cold? These things happen because of the “exchange of information” between our immune system and our mind. The mechanisms that regulate this crosstalk are not fully understood and might provide some very important information for humans and other animals.

Our research project aims at deepening our understanding of how immune cells (the sentinels of our body) influence our emotional wellbeing and how emotional wellbeing influence the immune response. We plan to investigate how both positive and negative emotions can impact the development of immune disorders and how by modulating the immune system we can improve our mood.

The results of our studies are intended to help to identify new treatments for both mental and immune disorders.

Mental disorders such as anxiety and Obsessive Compulsive Disorder (OCD) are chronic, relapsing psychiatric afflictions with a lifetime prevalence of 1–3%. The most effective treatments for mental disorders like OCD are antipsychotic (drugs used to treat psychotic disorders) and behavioural treatments. However, around 30% of the patients are resistant and unmanageable to pharmaco- and behavioural therapy.</td></tr>
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	In the case of immune disorders, a great deal of evidence have shown that patients suffering autoimmune diseases (diseases caused by immune cells produced against substances naturally produced in the body) show higher incidences of mental disorders compared to patients suffering other chronic pathologies. For instance, about **40%** of patients suffering multiple sclerosis have tried to **commit suicide** while more than **30%** of patients suffering autoimmune hepatitis also suffer form **schizophrenia**. The mood disorder therapeutics market is forecast to show growth steadily until 2017. This steady growth is primarily attributed to the increase in treatment-seeking behaviour and disease awareness. There are currently no treatments that have a dual beneficial effect on immune and emotional disorders.
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 benefit of our research are two folds: On one side, we aim to seek a better knowledge of the crosstalk between emotions, environment and the immune response testing new experimental models where emotional and immune response can be measured. The gaining of this essential information can be used in the future to inform policy-making agencies on the importance of these connections for the welfare of all animals; On another levels the results of these investigations will tell us what are the mechanisms by which we can improve or worsen our physical and mental conditions. These “endogenous (having an internal cause or mechanism) mechanisms” of functioning are most important because there are ‘drug-free’ i.e. they are not induced by synthetic drugs and are prompted by our own cells/tissues or lifestyle decisions. We think that the discovery of these new mechanisms of ‘healing’ can be important for the discovery of new therapeutic treatments that aim at improving our wellbeing. Our proposed program of research in experimental animals offers the unique opportunity to look at both sides of the coin (the immune and the emotional side) in the same controlled genetic background thus avoiding confounding factors found in humans

	such as social and economical factors that especially in this field would greatly influence the outcome of the research. In addition to this, the availability of animals genetically altered for a single gene and in specific cell types - an experimental condition not attainable in humans – presents the unique and fundamental advantage of assessing the specific contribution of that given gene in pathological conditions. The availability of these experimental models will thus represent a stepping stone towards the selective targeting of specific gene that might be involved in 'emotionally-driven immune disorders" or immune-related emotional diseases. Last but not least, we plan that the results of our studies will create an 'opinion shift' in the health sector and prompt us to adopt a more holistic approach to treat emotional and physical disorders. Indeed, we like to think that our results will prompt the design of clinical trials where the effectiveness of standard immunomodulatory or anti-inflammatory drugs might be significantly improved when it is associated with 'emotional modulators' such as massage therapies, art therapies, or just 'talk therapies' that improve the social and emotional state of patients. In this sense, improvement can be either a more effective therapeutic response or similarly a reduced dose of the drug; and hence a reduced incidence of side effects.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice as these are currently the species for which we have already gathered a wealth of very important information and historical background on systems regulating the immune response and the emotional wellbeing. We expect to use an average of 500 animals per month or about 6000 mice each year. These numbers have been generated in light of our previous experience and following the overarching ethos of our project to significantly reduce the use of animals to the minimum.
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	Mice will receive compounds that cause inflammation such as components of bacteria or chemical irritants. They might also undergo procedures such as the occlusion (blocking) of a vessel (to simulate human conditions such as stroke or ischemia). To mimic chronic inflammatory

end?	diseases such as rheumatoid arthritis or multiple sclerosis they will be injected with solutions that 'instruct' the immune system to attack the mouse's own tissue- hence the name 'auto' (meaning against itself)-immune disease for these pathologies. These procedures are likely to cause suffering from a number of ill effects such as pain, swelling, ulceration and paralysis. In the case of three of the procedures we are utilising (out of five in total), the severity will be moderate while only one will be severe. These mice will also be subject to conditions that impact adversely upon their emotional wellbeing. Procedures designed to create a state of anhedonia (the inability to experience pleasure from activities usually found enjoyable) or depression may lead to reduced feeding and a lack of self-care by individual mice. Since we will be investigating both positive and negative emotions, there will also be an equal number of mice for which we will try to improve their wellbeing and environmental conditions.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is as yet no comprehensive biological readout for an emotion. In turn all emotional responses involve an element of consciousness that cannot be effectively imitated in current cell based systems or computer models. As such a 'complete' animal with an intact and interconnected nervous and immune system is an intrinsic (belonging naturally) requirement of this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Efforts will be made to ensure the maximum amount of experimental data is acquired from each mouse used (without causing undue or excessive suffering to the mouse). One means by which this will be achieved is through obtaining both an emotional and immunological readout from every individual mouse used. The correct number of mice will be used for each experimental group and these have been drawn based on previous tests and experience using a number of statistical 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	Each technique used in this project has been validated by repeated use in published research as well as – in the case of the inflammatory models used – regular use by our lab. Care has been taken to select techniques that are considered the most

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	effective and ethical means of inducing a particular emotional or immune state. Systemic analgesic will be avoided because they might interfere with the inflammatory/immune reaction. For instance both innate and adaptive immune cells express receptors for opioid that are know to have a modulatory effect on these cells. As our project places a focus on inducing and observing emotional wellbeing in mice a significant effort will be made to ascertain the best means by which suffering can be avoided or at least alleviated in our experimental animals. Additionally our project will ascribe to the guidelines given by the NC3R^{S} for the basic requirements for good rodent housing and husbandry (https://www.nc3rs.org.uk/our-resources/housing-and-husbandry/rodents). These include for instance 1. The housing in stable, compatible groups - it is important to take into account sex, age, reproductive condition, familiarity, prior group housing experience when grouping the animals; 2. The use of enclosures designed to cause minimum disturbance to the animals; 3. The use of enough space for exercise, normal social behaviour (e.g. grooming, play) and the provision of environmental enrichment to help reduce the risk of social stress and aggression and allow the animals to fulfill some of their species-specific behaviours; 4. Gentle and frequent handling from early in life. A series of measureable endpoints and behaviours will be utilised to determine their physical and emotional state at any given time. If the perceived welfare state of any animal drops below a given limit it will be either removed from the experiment or terminated as context dictates.

Project 46	**Mice with human immune systems for drug discovery**	
Key Words (max. 5 words)	Human immunoglobulins, vaccines, mice, T-cell-receptor	
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)	Mice will be generated in which two types of cells white blood cells important in the immune system (B cells and T cells) will be humanized. To do this millions of base pairs of human DNA will be carefully inserted into the correct place in the mouse genome. These mice will make human antibodies and have human T cells. These mice will be used to identify human antibody drugs and to improve vaccine discovery for a variety of infectious diseases such as malaria and HIV.	
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 is directed towards drug development, specifically therapeutic antibodies and vaccines. Antibodies are some of the most effective and best selling medicines in the world. They are natural products which are safe, highly potent and very specific. Unlike most drugs, which are quickly removed from the body, antibodies can last for a month or more in circulation. Therefore, these can be administered monthly rather than daily. Recent examples of approved medicines are the drugs that encourage the immune system to attack cancer cells. These drugs are now curing cancer patients rather than just delaying death.	

What species and approximate numbers of animals do you expect to use over what period of time?	This is a five year project which will use mice only. Approximately 100000 mice will be used over the course of this project. Most of these animals (>90%) will be used in breeding experiments to establish strains. The numbers are large because there are many genes to humanize and several of these are huge, a million base pairs or more. More than 10,000 mice will be used in vaccine discovery efforts. The numbers are large, because in order to generate an effective vaccine, we must test many hundreds of candidates each in a dozen or more mice to obtain a reliable result.
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?	This project involves extensive genetic engineering, mouse breeding, immunisation and antibody isolation. Overall expected adverse effects will be small in both number and severity. Most adverse effects will occur during the generation of the genetically manipulated mice and are associated with developmental defects. When this happens, the affected foetuses die before birth, though some also die at birth or shortly after birth. The developmental defects are caused by the technical process and not a consequence of the genetic changes. The mice with human immune systems will be immunised, blood samples collected and when they have a good response to the vaccination tissues will be collected to isolate the antibodies and T cells. Although immunisation is also mild, occasionally the vaccine does cause a bad reaction, therefore this aspect has been assessed as moderate. However >99% of the mice will not experience any adverse effects. At the end of the experiments the mice will be humanely killed.
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 extremely complex and highly regulated. It produces molecules which attack foreign antigens. The immune system begins to develop before birth and continues through out adult life. The development and function of the immune system involves the interaction of many different cell types and migration of cells to a variety of sites in the body. It has not been possible to recapitulate such complex interactions *in vitro.*
2. Reduction Explain how you will assure	The numbers of animals used for this project are based on 30 years of experience in generating mice

the use of minimum numbers of animals	with altered alleles from genetically modified ES cells. We will continue to refine these methods, for instance by extensive pre-screening ES cell clones before starting any mouse work. We avoid large numbers of mice in breeding programmes by isolating and further manipulating ES cells, rather than breeding thousands of mice. One of the most important aspects in assuring minimum numbers is careful planning of experiments to generate just the right amount of data and technical competence. This will insure that the goals are reached with the absolute minimum numbers of mice. We will strive to maintain highly skilled personnel on this project. Finally, data tracking and integrity are important aspects in reducing animal. An animal tracking data base greatly facilitates this effort.
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 was selected for this project because genetic technologies have been developed in the mouse over the last 35 years to an extraordinary degree of sophistication and efficiency. This is based on the Nobel Prize winning embryonic stem (ES) cell technology but is also supported by the sequence of the mouse genome. The refinement of the experimental design and techniques mean that many fewer animals will be needed compared to reaching the same goals in another species. The immune system is conserved between mouse and human. Functional replacement of the mouse genes with their human counterparts has already been demonstrated. ***Minimizing welfare costs*** The replacement of the mouse immunoglobulin genes has resulted in full functional replacement, thus these animals are not be affected by this genetic change. Similar results are expected with the T cell genes. The other genetic changes contemplated in this project require that mice are unaffected by these additional genetic changes. Where possible we will search databases to ascertain the expectation for each gene that we manipulate before an experiment is conducted and if it is likely to be deleterious we will refine how it is manipulated to avoid welfare issues.

Project 47	**Regulation of the Immune Response in Tissues**	
Key Words (max. 5 words)	Immune response, infection, cellular immunotherapy	
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
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cells of the immune system play a crucial role in eliminating infected or cancerous cells from the body. However, immune cells sometimes fail to properly control infection and cancers, and can cause damage to healthy tissue. Consequently, improving the performance of a persons immune cells, or giving injections of new immune cells that function properly, are attractive approaches to treating disease. To achieve this, we first need to understand the factors that determine whether immune cells are protective, destructive, or ineffective. We believe that a key factor regulating immune cell function is their ability to migrate through complex tissues. For example, immune cells can become stuck in the dense tissue deposited around tumours, preventing them from reaching and killing the tumour cells. The purpose of this programme of research is therefore to understand how immune cell migration and function are regulated in complex tissue environments, with the eventual aim of identifying new therapeutic targets for the modulation of the immune response in disease. We will achieve this by studying how immune cells behave in tissues following infection, and how infectious agents can manipulate the way that immune cells migrate through tissues. For example, a parasite, *Toxoplasma gondii*, can invade immune cells, and modify their migration to enhace its own spread.	

	Understanding how it does this will have the dual benefits of helping us to understand how to therapeutically modify immune cell migration, and how to control *T. gondii* 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)?	This project will have two major benefits (1) It will help us to understand how immune cell migration is regulated in tissues, and how this influences the ability of the immune cells to function properly. Ultimately, this will allow us to manipulate immune cell motility for therapeutic purposes (for example cellular immunotherapy in cancer, or improved control of infection). (2) It will help us to understand how *T. gondii* manipulates the host immune response to enhance spread. These studies will reveal potential targets for the development of novel drugs and vaccines. *T. gondii* infection has serious consequences for human health. Reactivation of the parasite in immunocompromised individuals results in life-threatening toxoplasmic encephalitis, while infection during pregnancy can lead to miscarriage, stillbirth, or severe congenital defects. Treatment options are largely limited to sulfonamide and/or pyrimethamine-based compounds, which target the rapidly dividing tachyzoite stage, but not long-lived tissue cysts. Furthermore, evidence exists for increasing incidence of treatment failure, suggesting drug resistance may be developing, and no human vaccine is currently available. Consequently, new research aimed at the development of novel treatments and vaccines is required.
What species and approximate numbers of animals do you expect to use over what period of time?	Our experiments will be conducted in mice. We anticipate that we will use up to 6700 mice over a five 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 infected with pathogens, and the immune response studied. Some of these mice will also be administered agents that modify the immune response, so that we can understand how this affects control of infection. At defined time-points following infection, the mice will be euthanised by a schedule 1 method, before tissues are harvested for analysis. Wherever possible, we will perform this analysis when mice are experiencing mild or no clinical symptoms of disease. In some cases, mice will show moderate clinical symptoms of disease, which may include

	weight loss, diarrhoea, or poor grooming. These mice will monitored closely, and the experiment terminated if they approach pre-defined criteria that suggest these moderate clinical symptoms might be exceeded in the future.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The behaviour and function of Immune cells is controlled by the complex three-dimensional tissue environment in which they reside. However, our understanding of the precise molecular pathways involved is still in its infancy. At present, it is not possible to recapitulate the complex physiology of these tissues in an *in vitro* model. Animal models are therefore required to determine how the tissue environment influences the ability of immune cells to drive inflammation, or fight infection and tumours. Nevertheless, we have adopted sophisticated *in vitro* models to replace as much *in vivo* work as is compatible with achieving our aims. These recapitulate basic features of the *in vivo* tissue environment, and include the cultivation of mini organs or "organoids" in tissue culture dishes. However, these models still lack key features of the in vivo tissue environment, and we therefore occasionally require the use of *in vivo* models to test whether the pathways investigated in our *in vitro* experiments actually contribute to the ability of immune cells to control infection or inflammation at a systemic level. This is crucial if we are to translate our findings to the development of novel therapeutic strategies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible, we will use tissue culture techniques to address our research questions. Where animal experiments are required, we will perform power analysis calculations (in consultation with statisticians) to determine the minimum number of animals required for the experiment**.** The number of animals required to reach statistical significance will depend on the exact parameter being measured, but will be calculated to ensure at least an 80% chance of detecting a statistically significant difference at the 5% level. Typically, this will mean that we use 4-10 mice per experimental group.
3. Refinement Explain the choice of species and why the animal model(s)	A mammalian species is required for the study of the regulation of immune cell migration in tissues, and to accurately model human infections. Many of the

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.	functions of immune cells are shared between mice and humans, and mouse models have contributed greatly to our understanding of the human immune system. Furthermore, due to the wide availability of both immune modulators and defined genetically altered strains, they are the most useful species to interrogate the role of particular molecules or pathways in the immune response. Where possible, we will perform analysis at time-points that allow for adequate induction of an immune response, but that precede development of clinical signs of disease. This will minimise welfare costs to the experimental animals. We have extensive experience of monitoring and scoring schemes for detecting adverse effects or clinical symptoms in experimental animals. We will use these to determine when an experiment should end, again minimising welfare costs.

Project 48	**Inflammation and Immunity in Digestive Diseases**	
Key Words (max. 5 words)	Inflammation liver gut pancreas	
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)	The overall aim of the project is to test the hypothesis that targeting drugs against Stat2 (or the genes and proteins it acts on) can be used to treat inflammatory disease in the digestive system.	
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)?	Inflammatory diseases of the digestive system affect millions of people worldwide. Liver disease is the fastest growing cause of death in the developed world. Alcohol, obesity and viruses are the major causes of liver disease and each of these agents can trigger inflammation, which in turn drives liver scarring. Similarly, the development and progression of pancreatitis and inflammatory bowel diseases are governed by inflammation. The researchers' earlier work identifies an important role for Stat2 and they now wish to build on these findings to identify targets for drug treatments for us in patients with inflammatory digestive diseases.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. Approximately 8000 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?	In human diseases such as chronic liver disease, patients are asymptomatic until the late stages. In the same way we do not expect the models of inflammatory liver disease to cause significant harm to animals especially as we are not studying these late stages. The level of severity of all our experiments is expected to be 'moderate'. All animals will be monitored closely for signs of distress and if amnimals exceed the limits set in each protocol, then they 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	The complex interaction between the different components of the inflammatory and innate immune responses cannot be faithfully reproduced using cell lines or tissues. Human tissue cannot be readily sampled from the liver and pancreas, limiting the study of these diseases in patients. However, wherever possible, we will replace animal work with experiments using cells in petri dishes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will work with statistical colleagues to ensure that based on our early results, the experiments we design will use the minimum number of animals without compromising the quality of the data produced.
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 mammalian species of lowest sensitivity that are likely to produce satisfactory results. The models of the digestive disease we study are established in the mouse and are accepted in the scientific community as valid models for human disease. Not all animals will be subjected to the models of human diseases. For example, we will use methods that will be performed under terminal anaesthesia or that cause minimal suffering for experiments where are studying individual processes.

Project 49	**The role of circadian clocks in immunity**	
Key Words (max. 5 words)	Circadian, Inflammation, Lung, Glucocorticoids, Parasites	
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)	It is now widely recognised that that circadian (daily) clock controls multiple aspects of physiology, including immune function in both animals and man. Our recent studies and those of other laboratories show that circadian clocks located within cells of the immune system play an important role in determining the magnitude of immune response to inflammatory agents and disease causing organisms. Here, we address the key questions: How does the circadian clock control immunological responses? Our primary scientific goal is to define the genetic and biochemical mechanisms whereby the circadian clock couples to the immune system, as this will lead to novel therapeutic options.	
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 humans and animals, the magnitude and nature of immunological responses are strongly regulated by circadian clocks. Further, in humans, many diseases exhibit strong circadian presentation. It is therefore important to gain new knowledge of circadian mechanisms for the following reasons: 1) Insight into circadian mechanisms driving disease will allow future optimisation of drug treatment to maximise therapeutic benefit with minimal toxic side-effects. At present, most medicines are delivered	

	without regard to circadian time, with potentially deleterious consequences (see for example: Zhang R, et al (2014) A circadian gene expression atlas in mammals: implications for biology and medicine. PNAS 111:16219-24). 2) The work will potentially lead to the development of novel “chronotherapeutic’ options in which drugs targeting the circadian clock can be used to reduce the magnitude of inflammatory responses. 3) Our studies and those of others have major implications for the efficacy of immunization protocols in man, as major time-of-day effects are likely in the nature and magnitude of the antibody response. This has considerable public health implications.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 14,550
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 procedures employed under this licence are classified as mild or moderate severity. Adverse effects are minimal and relatively rare, but may include: 1. Poor recovery after surgery (e.g. implantation of a telemetry device or osmotic pump). 2. Respiratory distress after an immunological challenge targeted to the lung. 3. Subdued behaviour, laboured breathing, redness of feet/tail, due to allergic reaction after antigen sensitisation. Any animals exhibiting such adverse effects that do not rapidly subside, will be removed from the study and killed using a schedule 1 method. At the end of each experiment, animals will be killed by Schedule 1 method, or non-Schedule 1, including perfusion fixation (AC).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro cell-based assays cannot adequately model the complete array of inflammatory responses or address how systemic timing signals may modify these responses. Therefore, further in vivo work on living animals is required. In vitro assays do however offer a powerful model to test the action of novel anti-inflammatory compounds. We have successfully utilised cell lines (such as Human Bronchial Epithelial

	Cells) and exposed these to inflammatory challenge. However, in living animals and man, many different cell types are involved in producing an integrated immune response and for this reason, the use of cell-based assays is limited in value.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal husbandry Where we are using transgenic animal colonies, we consistently maintain numbers of breed stock and experimental mice to the minimum to achieve or goals. By regular tracking of breeding, rapid processing of samples for genotyping and excellent communication between our laboratory staff and the animal unit we minimise our animal usage. In vivo models Many of our in vivo models of inflammation have already been refined, and earlier work has established optimal dosing to achieve an effective inflammatory response in vivo. Thus there is a reduced need for studies to define responses to a range of doses. This is aided by our investment in new delivery systems for aerosolised agents (e.g. lipopolysaccharide), which will eliminate some animal-to-animal variation, leading to a reduction in group sizes in the near future (following consultation with our local statisticians). Sample analysis One of the major measurements made from immunostimulated mice will be their cytokine response (e.g. levels of cytokines and chemokines in blood and/or bronchoalveolar lavage (BAL) fluid). To further minimise animal numbers, samples will be analysed using multiplex analysis. This allows analysis of up to 23 cytokines/chemokines in a single small volume sample, significantly minimising the amount of samples needed. Over the last 12 months, we have changed the manner in which we analyse cell content of BAL fluid from manual cytospins to flow cytometry. This has enabled us to glean more information from each sample and has proven more consistent, and has enabled us to reduce group sizes. Additional cutting edge technologies are available to us at our institution, such as the nanostring nCounter system. This facility allows extraction of huge data sets from very small samples (i.e. single cells) and will reduce the number of experiments needed to generate significant information to advance our

	knowledge of this field. Archiving samples We routinely archive our tissue samples from in vivo experiments at -80°C and have built up a bank of samples over recent years. These can be accessed by others in the group and avoids repeating experiments unnecessarily thus reducing animal usage.
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.	Our studies will focus on the use of laboratory mice. This species offers unparalleled opportunities for investigations of the underlying genetic mechanisms involved in circadian timing and immune function, and essential prerequisite for targeting of pathways with novel drugs and therapies. We have established and validated colonies of mice that lack either a functional clock, or the receptor for glucocorticoids or clock genes, in different immune cells (e.g. macrophages and club cells). These are excellent model organisms for addressing key questions into clock control of immunity, which could not realistically be achieved in any other way.

Project 50	**Immune responses in Helicobacter infections**	
Key Words (max. 5 words)	Immune, responses, Helicobacter, infections	
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)	Inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis, are chronic inflammatory disorders of the intestinal tract, Recently published data suggest that up to 620,000 people are affected by these diseases in the UK alone. Treatments (medications, nutritional therapies, and surgery) remain unsatisfactory, and individual regimens are seldom effective for all patients, making investments into finding new therapies for IBD a high priority. Normally, healthy individuals live in harmony with their intestinal bacterial flora, but in patients with IBD, something goes wrong, and their immune system tries to fight off these microbes, leading to intestinal inflammation. The main objective of this project is to gain a better understanding of the interactions between our microbiota and the immune system in the healthy versus diseased intestine, as it may open up new avenues for therapeutic intervention for IBD.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	The primary potential benefit likely to derive from this project relates to new knowledge about the immunological mechanisms underlying intestinal inflammation. A secondary potential benefit relates to	

animals could benefit from the project)?	the identification of molecular targets at which new drugs could be aimed. Although not specifically designed to deliver new treatments to the clinic, application of the new knowledge about the immunology of intestinal inflammation may, in the longer term, make some contribution towards that goal. A third potential benefit relates to the 3Rs. More specifically, we envision that our work on developing a computational in silico model of colitis will in the longer term help reduce and refine animal work in the area of intestinal inflammation research.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use a mouse model of bacterial-induced intestinal inflammation. We estimate to use 440 mice/year 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?	We propose to give mice an intestinal bacterium, and depending on the mouse strain used, this bacterium will or will not induce intestinal inflammation in the host. In the former case, the inflammation will resolve with time so that round 3 months after bacterial inoculation, there is little or no signs of disease remaining. For mouse strains that develop intestinal inflammation, our experience over many years has shown that the animals appear normal while alive; they look healthy, they eat and drink as normal, and they do not loose weight. The only sign that a live mouse has ongoing inflammation is the presence of looser than normal fecal pellets in the cage. Because intestinal inflammation is present, the severity limit of this protocol is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro assays and cell culture experiments are an important component of research into IBD, and such experiments will — together with in silico approaches — be utilized in this project. However, the ability to manipulate immune and microbial factors, and to evaluate the pathological consequences in the intestinal tract following strategies aimed at preventing or reversing intestinal inflammation are still highly dependent on in vivo models. As it is not possible to perform studies in which immune responses to infection of human subjects are manipulated or examined in a controlled manner, it is necessary to use a whole-animal biological model for

	the studies proposed under this licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Previous experience with the types of experiments outlined in the application will help minimise the number of animals used. For analysis and assessment of experimental data, adequate statistical methods will be used. By efficient planning of experimental schedules, tissues from individual mice can be put to multiple uses. By storage of multiple samples from each experiment we will create a bank of tissues, which both reduces the repetition of experimental protocols and allows testing of new hypotheses without needing new experiments. Maximising analysis of individual mice also refines the analysis when there are inherent variations between infections.
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.	Choice of species: Mice are appropriate hosts for the present study because: a) Helicobacter species normally colonize their intestines. b) Numerous strains of mice are available with different deficiencies in their immune system, making them resistant or susceptible to helicobacter infection. c) Much is known about the murine immune system and how it can be manipulated, and reagents to perform such experiments are available (e.g., monoclonal antibodies). d) The responses of mice to helicobacter species have been extensively studied previously, and earlier published literature, including that of the applicant, will assist in interpreting these studies more effectively, permitting the efficient use of animals. General measures to minimise welfare costs to the animals: All animals will be kept in individually-ventilated cages with autoclaved bedding, autoclaved or acidified water, irradiated or autoclaved diet, and enrichment materials in a specific pathogen-free facility. Animal care and husbandry is provided by trained staff who check on all animals daily, and the facility also has access to a veterinarian. All experimental procedures have

	specified, well-defined “end-points”, e.g. symptoms of ill health such as failure to eat of thrive or a loss in body weight, and if an animal displays such symptoms, it will be humanely culled by a Home Office-approved method.

Project 51	**Perturbations of adaptive immunity in inflammatory arthritis**	
Key Words (max. 5 words)	Immune response, arthritis, cell migration, 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
		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)	Rheumatoid arthritis (RA) is a chronic, severe and debilitating disease that incurs substantial costs to the Health Service. The condition can have a major impact on the capacity to remain in employment unless the disease is treated early and intensively. Current therapies are only effective in 60-70% of patients, but therapeutic intervention at the very earliest stage of the disease can substantially improve outcomes. *PTPN22* is a gene that carries mutations known to be associated with a wide range of autoimmune diseases, such as RA, lupus and diabetes. It is one of the stronger genetic associations known. How these mutations contribute to disease susceptibility is poorly understood. The aim of this project is to study the immune response in animals that lack the *PTPN22* gene altogether, or in animals that carry the corresponding human mutation introduced into the mouse genome. Understanding how these mutations alter the immune response will give us important clues as to how, in at risk subjects, the immune system changes before the disease starts. This information will also help us to design new therapies that aim to restore immune health and perhaps prevent disease altogether.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	It remains unclear how *PTPN22* gene mutations contribute to disease. There are contradictory studies, some claiming that the gene works more efficiently, others suggesting less efficiently. It is	

animals could benefit from the project)?	essential to work this out to help understand why diseases such as Rheumatoid Arthritis (RA), lupus and diabetes develop in the first place. One way is to generate mice carrying the human mutation, and then conduct experiments to investigate how the mutation changes the immune system – not just in tissues and cells, but at a whole organism level, as we propose to do here. This offers real opportunities to identify in a systematic fashion what goes wrong before clinical disease becomes apparent. If we are successful, we will gain new insights into the cause of chronic inflammatory autoimmune diseases such as RA and lupus, with the possibility of developing targeted therapy capable of resetting the immune system.
What species and approximate numbers of animals do you expect to use over what period of time?	This project involves work that will be undertaken exclusively in wild type and genetically engineered mice. Based on our annual returns to date, we anticipate using no more than 1,000 mice per annum for the 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 level of severity? What will happen to the animals at the end?	Our protocols vary in severity from unclassified/mild to moderate, where adverse events are expected in experiments where arthritis is induced. Accordingly, pain and joint swelling from inflammatory arthritis will be the most common adverse effect, while the frequency of adverse events arising from specific procedures, e.g. minor surgical interventions, injections, administration of adjuvants or immunomodulatory agents is less predictable. Animals will be monitored throughout all steps of each protocol to determine the extent of the inflammatory response, pain or tissue damage and in turn time the termination of experiments to minimise suffering and harm. In such cases, 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	The availability of cells and tissues from human subjects carrying PTPN22 mutations are limited, and many of the studies required to understand PTPN22 function would not be possible in man. Many of the immune cell subsets we wish to study are also found at very low frequency in human blood and tissues. While we have made every effort to exploit the availability of human samples, the provision of *Ptpn22* mutant mice that have the corresponding mouse mutation introduced into the mouse genome will allow us to define how this mutant changes the immune response in ways that contribute to disease

	in a range of different immune cell types (including rare cell subsets derived from lymph nodes and spleen), in tissues and at a whole organism level (e.g. by studying disease), in ways that would not be possible in man. Thus, the availability of *Ptpn22* deficient and mutant mice provide a unique and abundant source of immune cells for both *in vitro* and *in vivo* studies that would not be possible in man.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals required for this project have been considered in some detail, based on our own experience, as well as on information from the literature. In many experiments we will be able to generate and expand cells in vitro (eg from bone marrow, lymph node or spleen), meaning that we can limit the number of donor animals required for such studies. Other examples of reduction are illustrated below: *(a) Induction of arthritis*: We will use arthritis protocols that have been optimised using a strain called C57BL/6. In this strain mice, the disease occurs at lower incidence (40-50%) is less severe, but more chronic. In our experience, 10 mice per group has been sufficient to detect the differences we predict. In another arthritis model, where we inject serum, disease frequency is substantially higher (> 90%) and the severity can be modified according to the titre of arthritogenic serum administered. For these experiments we can use fewer animals (eg 5-7) per group. *(b) Cell migration and live imaging studies*: We have adapted protocols to study migration of cells *in vivo* to reduce numbers of animals in several ways. Firstly, we have defined the minimal number of cells that allow us to track them after adoptive transfer into recipients, thereby minimising the number of donor mice required. Secondly, we now routinely generate a 1:1 mixture of wild type and mutant cells and co-inject them into recipient animals. This reduces numbers of recipient 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 general measures you will take to	We have adapted our experiments so that we can study inflammatory arthritis over a period of days (serum transfer model) or 3-4 weeks (for other types of arthritis induction). Where appropriate, analgesics will be administered, when clinical signs dictate. The severity limits will be capped such that any animal with a clinical score or 8 or more, or a score of 3 in

minimise welfare costs (harms) to the animals.	more than one paw, will be killed humanely. Measures such as softer bedding and more easily accessible food will be taken to minimise suffering. If maximal joint scores are achieved for any particular animal, that mouse will be killed humanely. Many of our other protocols involve procedures undertaken before the onset of disease. This will substantially minimise the number of mice with active disease in our colonies. We do not plan to undertake any protocols categorized as severe.

Project 52	**Regulation of immune responses in health and disease**	
Key Words (max. 5 words)	Inflammation, cytokines, infection, repair, autoimmunity	
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 critical role in protection against microorganisms that cause disease. Immune cells are essential to maintain the integrity of barrier sites such as the skin, the lung and the intestine. Furthermore, the functioning of the immune system is also influenced by environmental factors, which can be external pollutants as well as endogenous factors, eg dietary components. The aim of this work is to gain a deeper understanding of how the immune system functions to fight infections and how it protects barriers. On the other side we want to find out what goes wrong when the immune system makes inappropriate responses leading to autoimmunity or allergy. The immune system has been widely studied for many years. As a result we know that there are many different types of white blood cells involved in the response. To mount an appropriate immune response, these cells need to detect pathogens, interact with each other and with other cells of the body, and orchestrate a complex series of events culminating in the elimination of the pathogen. At the same time, the immune system needs to avoid mounting inappropriate responses, as happens in autoimmune or allergic diseases. Despite a large body of knowledge, we still do not understand many of the molecular mechanisms that underlie a correct immune response, and what goes wrong during	

	dysregulated inflammatory responses. The complex interactions between components of the innate and adaptive immune system with surrounding tissues and organs require whole body model systems as culture systems can not adequately mimic the complexity of tissue interactions that shape immune responses. The mouse is the most appropriate species for these studies because, as a mammal, its immune system is very similar to that of humans, and because of the extensive genetic modification technology that is available for the mouse, and the wealth of pre-existing data and methodology will maximise the net benefit from these studies.
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 research output from this project will lead to a better understanding of the function of the immune system. This will be applicable to human health through design of better therapies for infectious and autoimmune diseases
What species and approximate numbers of animals do you expect to use over what period of time?	We will breed approximately 22000 mice over a period of 5 years. Due to the complexity of genetic alterations we need to assess, the breeding of genetically modified mice will generate many genotypes that are not usable in functional analysis. Mice undergoing procedures will be in the range of 12000 over the 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 work will use mice carrying genetic alterations that inactivate or change the function of specific genes coding for proteins of interest. We will study the effects of these genetic changes on the function of immune cells, in particular in barrier organs such as skin, lung and intestine. In parallel, *in vitro* methodology will be used to investigate molecular details of cell functions and signalling pathways identified in our *in vivo* models, thereby reducing the number of mice that undergo procedures. The large majority of mice used in this project will suffer no adverse effects. A proportion of mice will be infected with pathogens that will elicit inflammatory immune responses and may lead to moderate symptoms. A small minority will develop autoimmune disease, typically reaching a moderate level of severity. Numbers of animals will always be chosen to be the minimum compatible with sufficient statistical power to generate meaningful results. At the end of procedures mice will be killed by a Home Office approved method.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The aim of the work in this project is to gain a deeper understanding of molecular mechanisms of the role of different proteins in controlling the function of immune cells, and their communication with cells outside the immune system and with environmental triggers. The complex interactions between immune cells and surrounding tissues that shape immune responses make it impossible to mimic these under artificial in vitro culture conditions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers of animals will always be chosen to be the minimum compatible with sufficient statistical power to generate meaningful results. In some cases we can reduce the number of experimental mice by using imaging technology that allows studying a cohort of mice over several time points without the need of killing them for analysis.
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 achieve our goals we propose to use the mouse as the model organism for several reasons. Genetic modification techniques are well-established in mice, their immune system has been intensively studied and it bears extensive similarities to that of humans. There exists a vast array of reagents facilitating the study of the immune system in contrast to the situation in other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this programme to the same extent as the laboratory mouse. Most of our procedures are mild or moderate and close monitoring of mice treated with any intervention will make sure they are not suffering excessive adverse effects. Mice will be housed in cages with environmental enrichments that allow them to burrow or hide in red plastic shelters (which appears black to them but allows researchers and animal technicians to observe them). This would maximise their wellbeing even under experimental procedures.

Project 53	**Neutrophil function in infection and disease**	
Key Words (max. 5 words)	Inflammation, cytokines, infection, autoimmunity, neutrophils	
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)	Neutrophils are immune cells that are critical for protecting against infection. They are armed with an array of antimicrobial factors whose deployment is tightly regulated. Misregulation of neutrophil function can lead to immune deficiency or autoimmune disease. Neutrophils kill pathogens by ingesting them and attacking them with antimicrobials intracellularly. In addition they release large web-like structures calls neutrophil extracellular traps (NETs) that trap and kill microbes extracellularly. We made breakthroughs in showing that NETs are important in protecting us against infection by large pathogens such as fungi. However, a wealth of emerging work has also shown that NETs can be detrimental for health if their release is not tightly regulated or are not effectively cleared. The mechanisms by which NETs cause pathology are only now being uncovered. Furthermore, it is becoming increasingly apparent that neutrophils and NETs play key roles in regulating inflammation during human disease. The bulk of this proposed work aims at elucidating the mechanisms regulating NET formation and clearance. In addition we will investigate the molecular mechanisms that are involved in neutrophil-mediated modulation of inflammation during immune responses to infection and inflammatory disease. Finally, we will also investigate mechanisms that enable certain bacteria to evade immune protection strategies in the gut.	

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)?	By understanding the basic mechanisms of neutrophil-driven pathology, the findings generated from these projects will allow the identification of new targets for the development of therapeutics to treat human diseases for which there are no cures and treatments are only palliative such as rheumatoid arthritis, cardiovascular diseases, autoimmune disorders, cystic fibrosis, immune deficiencies and indirectly various types of cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We will breed approximately 15000 mice over a period of 5 years. Due to the complexity of genetic alterations we need to assess, the breeding of genetically modified mice will generate many genotypes that are not usable in functional analysis. Mice undergoing procedures will be in the range of 11000 over the 5-year period. We anticipate that less than 30% of our genetically altered animals will not have the right genotype to be used in experiments.
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?	A proportion of mice will be infected with pathogens that will elicit inflammatory immune responses and may lead to moderate symptoms. A fraction (10%) of these animals may develop more severe symptoms under higher doses of microbial infection. A minority of the animals will develop autoimmune disease, typically reaching a moderate level of severity. Numbers of animals will always be chosen to be the minimum compatible with sufficient statistical power to generate scientifically sound results. At the end of procedures mice will be killed by a Home Office approved method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In nearly every study we use human primary cells to address how neutrophils function in isolation. However, to evaluate the significance of these findings in disease, we need to complement these studies with well-characterized, in vivo experimental models of human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers of animals will always be chosen to be the minimum compatible with sufficient statistical power to generate meaningful results. We will conduct small pilot experiments to optimize the conditions and size of experimental groups where needed and conduct biological replicates with smaller groups to gain higher statistical power from a total smaller number of mice undergoing procedures.

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.	Genetic modification techniques are well-established in mice, their immune system has been intensively studied and it bears extensive similarities to that of humans. There exists a vast array of reagents facilitating the study of the immune system in contrast other organisms. To our knowledge no other species of lesser sentience can fulfil the requirements of this programme to the same extent as the laboratory mouse. Most of our procedures are mild or moderate and close monitoring of mice treated with any intervention will make sure they are not suffering excessive adverse effects. Mice will be housed in cages with environmental enrichments that allow them to burrow or hide in red plastic shelters (which appears black to them but allows researchers and animal technicians to observe them). This would maximise their wellbeing even under experimental procedures.

Project 54	**NF-κB and MAP kinase signalling in immunity and cancer**	
Key Words (max. 5 words)	Autoimmunity; inflammation; cancer; mechanisms; therapy	
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)	An understanding of how immune responses are regulated at the molecular level is critical for the development of novel therapies to treat autoimmune and inflammatory diseases, such as inflammatory bowel disease, psoriasis, rheumatoid arthritis and atherosclerosis. Current treatments for such diseases are only partially effective and have multiple side effects. My laboratory researches key regulatory proteins that control activation of cells of the immune system. We plan to test whether blocking the activity of these proteins ameliorates the development of a number of autoimmune / inflammatory diseases, using mice carrying genetic alterations in these target proteins. Positive results would suggest that the regulatory proteins could be targeted pharmacologically to treat autoimmune / inflammatory diseases in humans. We will also test whether regulatory protein inhibition prevents effective immune responses to pathogens, which might be an undesired effect of drug targeting. A second aim of this project is to investigate the role of the regulatory proteins in the development of lung cancer and colitis-associated gut cancer. These experiments will use established pre-clinical mouse models of human disease. The results of these studies may identify novel approaches for cancer therapy and also define the effects of immune cell activation on cancer initiation and progression.	

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 our basic scientific understanding of autoimmune / inflammatory diseases and cancer. This may provide insights into new clinical strategies for the therapy of these significant human diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will only use mice in this project and a maximum of 10,000 animals will be used per year over the 5 year term.
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?	Our studies on the role of regulatory proteins in immune responses are likely to produce mild or moderate levels of severity. Mice will be studied under steady state conditions to study the effects of genetic changes on the development, survival and function of immune cells. The immune system of a proportion of mice will be challenged with model antigen or pathogen. For the majority of cases, no significant adverse effects are expected. A small minority will develop autoimmune disease, typically reaching a moderate level of severity. Numbers of animals will always be chosen to be the minimum compatible with sufficient statistical power to generate meaningful results. Mice will be killed by a Schedule 1 method if they appear hunched, immobile after touch or display other non-transient moderate clinical signs. Tumour prone animals will develop cancers that will eventually cause their death if there is not further intervention. To minimise suffering for these animals, stringent checks will be in place to ensure that mice are culled as soon as signs of suffering or distress as a result of their cancer are detectable. Only tumour bearing animals that are healthy will be subjected to non-invasive imaging to address the experimental aims of this programme. Very rare instances may occur where rapid progression of the cancer could result in death from the disease before any signs of suffering were detectable, despite stringent monitoring. It is expected that the rate at which this would occur would be no more than 5% of the animals bearing tumours in internal organs. Our aim is that animals will be culled at the end of the defined experimental period and before severity limits are reached.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In an immune response, multiple cell types interact with each other and with other cells of the body, and orchestrate a complex series of events culminating in the elimination of the pathogen. Similarly, aberrant activation of the immune system in autoimmune or allergic diseases involves a complex interplay between multiple different cell types. The complex interactions between components of the innate and adaptive immune system with surrounding tissues and organs require whole body model systems as cell culture systems cannot adequately mimic the complexity of tissue interactions that shape immune responses. The mouse is the most appropriate species for these studies because, as a mammal, its immune system is very similar to that of humans, and because of the extensive genetic modification technology that is available for the mouse, and the wealth of pre-existing data and methodology will maximise the net benefit from these studies. While valuable studies of human cancer can be performed using tumour material, the mechanistic understanding of cancer pathogenesis requires the use of living animals. Cancer development and spread involves a plethora of interactions between cancer cells and their surrounding cells, governed by multiple signals originating from both the immediate environments and distant sites. It is only possible to investigate these factors in living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have extensively used cultured cells to delineate the role of regulatory proteins in the activation of immune cells. These studies have allowed us to define a limited set of hypotheses that merit testing in animal models. Mouse breeding experiments have been planned in detail with experts in statistics and animal breeding. We will perform pilot experiments using a small number of animals per group are used for genotype comparisons. Depending on the results obtained, we will then proceed to perform larger cohort studies to determine if the observed difference is statistically significant. We will always ensure that the minimum numbers of animals are used to obtain statistically meaningful results. Mouse colonies will be actively managed to ensure that the basic principles or mouse breeding will be adhered to and only the minimum number of animals required for experiments are generated. Transgenic lines will be frozen down wherever possible rather than keeping in breeding

	stocks. In addition, the use of in vivo imaging methodologies to study cancer development greatly reduces the number of animals needed compared with end-point assays, as each mouse can be followed over time and inter-mouse variability is internally controlled for.
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 species for our studies as its mammalian immune system is very similar to that of humans. In addition, extensive genetic modification technology is available for the mouse, and the wealth of pre-existing data and methodology will maximise the net benefit from these studies. The mouse cancer models we will use are internationally regarded as the best available for the accurate modelling of the human disease, for example by the US National Cancer Institute (http://emice.nci.nih.gov/aam/mouse). Only by allowing tumours to develop in these mice can we address the importance of the molecular interactions that we are seeking to investigate in this setting, which may lead to the development of novel therapeutic interventions. In the cancer models, we will use transgenic mice in which mutations are induced specifically and conditionally in the cell population of interest. These mice should not display a phenotype until the mutation in the target gene is induced. The severity of the procedures will be limited by ensuring that animals are killed as soon as any overt signs of autoimmune disease or cancer can be seen, and that mice are rigorously monitored for signs of suffering or distress at all times. Live imaging of the animals used in cancer models, such as by CT scanning, will be a major feature of these experiments which will allow improved data collection from smaller number of animals; this will be carried out under anaesthesia to minimise distress to animals.

Project 55	**The Production of Antibodies**
Key Words (max. 5 words)	Antibody, Polyclonal, Monoclonal, Immunogen, Antigen
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 addressed)	The objective of this licence is to provide a service for the production of antibodies for both the medical research community and diagnostics manufacturing industry within the UK and Europe. Antibodies are produced by the immune system of a living organism and play an integral role in Biology in terms of their ability to fight infection by a host of organisms deemed foreign to self, their ability to detect life threatening disease and their use as critical tools in the areas of research and medicine, including basic research of cells and their function in disease, diagnostic technology and therapeutic medicine development.
What are the potential benefits	Antibodies routinely help scientists to research the

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	function of both healthy and abnormal cells in disease, by the detection of proteins within the cell at various stages of its development. This is routinely utilised when researching disease and its prevention. In the field of diagnostics antibodies play a critical role in the detection of disease in a clinical environment. This can allow for the rapid diagnosis of life threatening disease and assist in providing clinicians (clinical Scientists and Doctors) with specific information in terms of the most appropriate course of treatment to follow, thus preventing death. The use of antibodies in therapeutics is a fast developing area of medicine, with the use of antibodies as constituents of direct medicine in order to treat various diseases including cancer, auto-immune disorders and infection.
What types and approximate numbers of animals do you expect to use and over what period of time?	Rat 3,500 (5 Years) Mouse 6,500 (5 Years) Guinea Pig 2,500 (5 Years) Rabbit 12,500 (5 Years) Chicken 750 (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 happen to the animals at the end?	The production of custom antibodies requires the use of live animals, to which a substance, called an antigen, is introduced to produce an immune response. To assist in the development of an immune response to an antigen a substance known as an adjuvant can be used in conjunction with the antigen to assist in the further stimulation of the immune system and subsequent production of antibodies. Some adjuvants which are very effective in stimulating an immune response can cause tissue reactions in the animals at the site of injection, therefore the use of these adjuvants is carefully controlled, with any reaction being closely monitored. Subsequent blood samples will be taken from an animal in order to test the level of antibodies being produced within the animal. These blood samples will be taken from an appropriate collection site on the animal such as veins/arteries and as such can (but rarely) lead to the formation of bruising and slight skin damage. When raising antibodies against DNA, special technology has been developed to do so. This technology involves the use of DNA coated gold particles which are introduced to the animal via bombardment of the skin with pressurised gas. This procedure is carried out under general anaesthesia and has minimal associated effects, which can include slight redness of the skin at the site of

	inoculation. The production of antibodies against bacteria requires the inoculation of a bacterial liquid(without adjuvant) direct into the blood stream. This methodology can result in the loss of animal body weight and (rarely) the onset of symptoms that appear similar to that of an allergic reaction e.g. laboured breathing, reduced mobility and redness of the eyes with associated light sensitivity. Upon reaching a desired level of circulating antibody to an antigen an animal will be moved forward for exsanguination where animals are given an anaesthetic from which they are not allowed to recover and their blood is collected to provide the antibodies. When this has been done the animal is humanely killed and further tissues may be collected for scientific use. Although significant adverse signs within any animal used for the production of antibodies are not expected full veterinary attention will be provided should there be any unexpected consequences of any procedure carried out. All animals used for the production of antibodies under the authority of this licence are subject to well defined humane endpoints, which if experienced will result in the animal being removed immediately from the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-protected animal alternatives	At the time of writing this licence there are no alternative (non-animal) methods for the production of blood serum containing a wide variety of antibodies to various targets or the production of specific (monoclonal) antibody secreting cells.
2. Reduction Explain how you will ensure the use of minimum numbers of animals	The use of appropriate species and methodology will ensure the production of better quality and a higher number of antibodies, therefore reducing the number of repeat production programs required where use of additional animals would be needed. Our expertise and experience in this area allows us to provide guidance on best practice from the beginning, this includes the selection of appropriate species based on the substance to which the antibodies are to be raised against, the way in which the substance will be introduced to the host and the schedule of inoculations to be followed. With all of these considerations we can ensure that the minimum number of animals are used for each and every project undertaking.

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.	A variety of species (Rabbit, Guinea Pig, Rat, Mouse & Chicken) will be made available for the production of antibodies. Selection of a specific species , from one of the above will be made following the careful consideration of a number of factors, both ethical and scientific. Our experience in this field allows us to make ethically sound decisions based on knowledge and expertise as well as ensuring the highest levels of care and attention are afforded to all animals utilised in the production of antibodies. Our production protocols are designed with the principles of minimal severity and are under constant review to ensure best practice is followed at all times, whilst also keeping abreast of new and refined techniques/technology utilised in the field of antibody production.

<table>
<tr><td>Project 56</td><td colspan="3">Understanding how vaccines work</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Vaccine, Infection, Natural Killer (NK) cells,
T-cells, Ageing</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>Yes</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Vaccination induces immunity in some people and can generate long-lived immune memory. Our principal aim is to test the hypothesis that Natural Killer (NK) cell activation can mediate post-vaccination immunity.

Our main project objective is to characterise the role of NK cells in the generation of vaccine-induced immunity during and after vaccination against a range of pathogens, including influenza virus. We will test this objective using a strain of mice in which NK cells can be specifically depleted at any time with no additional adverse effects and subsequent recovery of NK cells within 14-21 days. Utilising this strain, and others, we will attempt to dissect the immunological pathways by which NK cells are activated during, and contribute to,</td></tr>
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	vaccine-induced immunity NK cells have been shown to change dramatically with age in humans. As NK cells may mediate post-vaccination immunity the role of NK cells in vaccination with age is an important consideration. We will therefore characterise the effect of age on acquisition and maintenance of vaccine-induced immunity.
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)?	Vaccination is the most sustainable and cost-effective way to reduce the global burden of infectious disease. New vaccines are urgently needed for complex infections including malaria, HIV and TB, whilst current vaccines may lose efficacy over time from waning immunity, and many vaccines work sub-optimally in aged populations. Rational design of new and improved vaccines might be informed by comparison with existing effective vaccines but these were, in the main, developed empirically and we have a very incomplete understanding of how they work. Expensive and time-consuming clinical trials remain the only way to evaluate vaccines for most human diseases; correlates of protection – which might allow triage of candidate vaccines – are lacking in most cases. Vaccination and challenge experiments will provide a thorough understanding of the role of NK cells following vaccination including the very first functional and observational characterisation of NK cells after vaccination and the first quantitative assessment of their contribution to post-vaccination immunity. Our experiments may reveal NK cells as important contributors to post vaccination immunity and reveal the most effective vaccination strategies to induce them. If so, this will provide entirely new biological indicators (biomarkers) of effective vaccination and may inform the development of more protective vaccines. Our studies will also provide an insight into the impact of ageing on vaccine-induced immunity. With an ever increasing life expectancy and

	increasingly elderly population it is essential to understand how vaccine immunity is maintained or lost throughout life and to identify appropriate vaccine formulations that will be more effective at protecting older people. It is widely recognised that older individuals respond less well to new vaccines (such as seasonal influenza vaccine) than do younger people but the explanations for this are unclear. Our hypothesis, that NK cells contribute to vaccine-induced immunity but that the highly differentiated NK cells that accumulate with age are less able to mediate this effect offers one potential explanation for this phenomenon and suggests that new
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse only with a maximum of 5000 animals 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 principal aim of this project is to test the mode and action of vaccines and therefore we do not expect that many mice will progress to a level beyond moderate suffering (i.e. it is not necessary for a severe state to be reached in order for the outcome of the experiment to be assessed). However, to study the requirements for protection from infection we recognise the risk that some mice may progress to this state on some occasions.
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 effectiveness of a vaccine, with or without infection, it is necessary to conduct these experiments using animals to extrapolate mechanisms of efficacy in humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical advice has been sought to minimise within group variation and the number of mice per group needed to detect any given signal. The optimal protocol for each experiment (e.g.

	timing/dosing/number) will be determined in pilot studies; where possible individual animals will be monitored over time in order to minimise the number of experimental groups required and to minimise the effects of inter-individual variation Where multiple outcomes are possible, or where a complex design is required, we will consult with our project statistician.
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. Multiple in-bred strains of mice will be used for experimental studies to minimise variation within and between experiments. We will also collaborate both internally and externally to utilise the wide range of resources and skills available to help refine our experiments. Full literature reviews will be conducted prior to the use of any new biologic or technique to ensure best practice. Imaging and pilot studies will also progressively enable us to refine our experiments further reducing animal usage.