Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 24

Projects with a primary purpose of Basic Research into the Immune System

Title and Key Words

1. **The study of immune-mediated proteases in tissue inflammation**
    - immune-mediated, proteases, tissue inflammation, therapy

2. **Prevention and diagnosis of infectious diseases**
    - Neisseria; Chlamydia; Norovirus; vaccines ; diagnostic reagents

3. **Autoimmunity in immune-privileged environments**
    - Multiple sclerosis, uveitis, autoimmune disease

4. **Leucocyte migration and immunity**
    - Leucocytes, blood vessels, tumours, viruses

5. **Prevention of bacterial meningitis**
    - Vaccines, bacteria, pathogenesis, meningitis

6. **Antigen-specific immunotherapy**
    - Autoimmune, Multiple Sclerosis, T-lymphocyte, Gene, Immunotherapy

7. **Pathogenesis and control of respiratory diseases**
    - Ruminant disease; respiratory disease

8. **Role of Innate Immunity in diseases of the CNS**
    - Immunity, Brain, Complement, Dementia, Inflammation

9. **Pattern Recognition Receptors in Bone Remodelling**
    - Bone, immunology, receptor

10. **Strategies to promote foreign transplant survival**
    - Transplantation, GVHD, T cells, iNKT cells, Treg

11. **Understanding the role of FcyRIIB in health and disease**
    - Antibody, immune-regulation, infection, autoimmunity, kidney-injury

12. **Studies of invasive fungal and bacterial infection in immunocompromised murine hosts**
    - Bacteria, fungi, immunocmpromise, transplant

13. **Host and parasite genes involved in rodent malaria**
    - Infectious disease, malaria, parasite-host interactions, Plasmodium genetics

14. **The regulation of innate immunity by the microbiota**
    - Innate immunity, infection, microbiota, vaccine

15. **Mucosal and parenteral vaccines for tuberculosis**
    - Tuberculosis, local immunity, vaccines

16. **Studies of antigen presenting cellsin vivo**
    - Dendritic cells; immunotherapy

17. **Basic and applied aspects of T cell immunity**
    - Adaptive immune system, T cell receptor, tumour, antigen

18. **Antibody production in rodents**
    - Antibody immunisation

19. **Adjuvant effects of lipid-coated particles**
    - Adjuvant effects: lipid-coated

20. **Antibodies research, diagnostics and therapeutics use**
    - Monoclonal, polyclonal, antibodies

21. **Understanding perturbation of innate immunity in vascular inflammation**
    - Vascular disease, inflammation, innate immunity

22. **Macrophage control of injury and repair**
    - Macrophage, kidney, inflammation, treatment

23. **Role of Fat Associated Lymphoid Clusters in Metabolism and Immunity**
    - Immunity, inflammation, metabolism, obesity

24. **Immunological defence against bacterial pathogens**
    - Tuberculosis, melioidosis, immunity, vaccines

25. **Host and bacterial factors interactions in tuberculosis**
    - *M. tuberculosis*, mouse,

26. **Understanding the role of signalling molecules in immune cells**
    - Immunology, signalling, gene expression, autoimmunity

27. **Identification and characterisation of haematpoietic stem cells**
    - Blood, transgenic, stem-cells, therapy

28. **Role of Pattern Recognition Receptors In Immunity**

- Immunity; Infection; microbes; autoimmunity

29. **Production of potent monoclonal antibodies**
    - Hybridoma, monoclonal, antibody, therapeutic, reagent

30. **Therapies for Infectious Disease and Cancer**
    - Cancer, influenza, tuberculosis, vaccine

31. **Murine models of innate and adaptive immunity**
    - Immunity/autoimmunity/bacteria/sepsis

32. **Service licence for Antibody Production**
    - Service Licence, Antibody production

33. **Antibody production to Novel Antigens**
    - Monoclonal Antibody

34. **Immunoregulation during parasitic helminth infection**
    - Parasites, Immunity

35. **Immunopathology and Immunotherapy of Hepatitis B Virus Infection**
    - Chronic hepatitis B virus infection, T cell therapy,
      Liver fibrosis, NK cell modulation, Hepatic tolerance induction

36. **Production and function of blood cells**
    - Red blood cell, T-cell, thymus, immunity, anaemia

37. **Investigating the immune response in the oral cavity**
    - Immunity, inflammation, infection, oral

38. **Using zebrafish to understand inflammation resolution.**
    - Neutrophil, macrophage, inflammation, innate immunity, zebrafish

39. **Initiation and maintenance of immune responses**
    - Immune response, vaccination, autoimmunity, tolerance

40. **Methods to enhance or suppress immune responses**
    - Vaccine, autoimmune disease, cancer

41. **Immunomodulation by helminth parasites**
    - Parasite, immunology, immunomodulation, therapy

42. **Rodent models of Disease**

- Genetically altered, breeding, disease models

43. **Natural variation in immunity in wild rodents**
    - immunopathology, infection, immunology, ecology, zoonosis

44. **Lymphatics and cell trafficking during inflammation**
    - Lymphatics, Cell migration, Inflammation

45. **Roles of monocyte/macrophages in metabolic and inflammatory diseases**
    - Immune system, macrophages, monocytes, development, inflammation

46. **Murine models of human haematopoiesis, therapy and disease**
    - Gene therapy, haematopoiesis

47. **Mechanisms of cellular guidance in vivo**
    - Leukocyte, neutrophil, chemoattractant, cell migration, chemotaxis

48. **Pathology of chronic inflammatory disorders**
    - Arthritis, Treatment, Alleviation, Inflammation.

49. **Exploiting endogenous tissue protection in inflammation**
    - Inflammation; Tissue Protection; Drug Discovery.

50. **Oral microbiology in health and disease**
    - bacteria, gum disease, inflammation

51. **Development of Novel Mucosal Vaccines**
    - Vaccine, probiotic, TB, influenza, Clostridium difficile

52. **Innate Lymphoid Cell functions in vivo**
    - Immune responses ,T cells, Innate Lymphoid Cells

53. **Antigen presentation by DCs**
    - Immune responses, infection, vaccines.

54. **Multigene families, immunity and virulence in malaria**
    - Malaria, Plasmodium, immunity

55. **Production of Ebola virus antigen polyclonal sera**
    - Antibodies, recombinant proteins, vaccine

56. **Studies on the immune system and disease resistance of fish**

- Fish, immunity, health, disease resistance.

57. **Development of a vaccine and preclinical drug evaluation model for HIV based on humanised mice**
    - Humanised mice, HIV, vaccine, cure

<table>
<tr><td>Project 1</td><td>The study of immune-mediated proteases in tissue inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>immune-mediated, proteases, tissue inflammation, therapy</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>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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Inflammatory bowel disease is an example of chronic inflammatory disease that affects 1 in 250 people in the UK. The cause is unknown and there is no cure. Crohn’s disease is a particularly vicious condition since the inflammation in the bowel wall may eat through into adjacent bowel or even exteriorise on the abdominal wall, leading to a fistulous tract between the skin and the gut. Crohn’s is also associated with inflammatory conditions affecting multiple organs, the gut, skin and bone. There is a tendency to try to look for “causes” of these diseases. Especially in the gut, there is a natural tendency to think that things we eat must be responsible for gut diseases. However the diseases are often complex and may be multi-factorial in origin with many end-stage presentation. What these diseases however have in common is</td></tr>
</table>

	that they are caused by an over-active immune system and high protease production. Whilst there have been some successes in identifying therapies which will reduce symptoms and increase standard of living, the current treatments are not effective and patients often relapse (symptoms re-activated) after remission (symptoms disappear) especially under stressful conditions. When colitis lasts more than 8-9 years, there is an increased incidence in inflammation associated colorectal cancer. Although there has been a lot of progress, there is still much that we do not know about the mucosal immune system in health and disease. We do not know what events trigger the hyperactive immune response which leads to the increased production of protease and chronic gut inflammation hence tumorgenesis. We do not know why the environmental factors such as stress perpetuates chronic inflammation in the gut also cause relapse. We do not know how systemic inflammation could accelerate ageing. The gut immune system is designed to recognise and eliminate pathogens, but for some unknown reason, when the immune system is dys-regulated, the immune system recognises the normal flora as a pathogen. We also do not know why and how patients who have Crohn's disease have inflammatory conditions affecting the other organs.
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 the work and its outcomes will be in terms of expanding the knowledge base through publications and conferences. This will also lead to improved understanding of basic mechanisms and pre-clinical aspects which may throw up therapeutic targets, as well as the pre-clinical evaluation of strategies to help patients with inflammatory diseases and other extra-organ conditions such as that in brain, joint, skin, eye and liver.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use about 2500 mice (GA and wild type) for the entire project. In general, GA mice are more easily available than other groups of animals so that we could look at any genetic effects on the over-active immune system in inflammatory

	conditions.
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 inject substances into the colon or the animals ingest substances to induce colitis. The protocols have been characterised and published extensively in high impact factor scientific journals. The procedures are well refined to minimise adverse effects and suffering to the animals. The first sign of an adverse effect can be loss of body weight and the severity of inflammation can be ranked using a scoring system. We therefore terminate the experiment if body weight falls below a specified percentage of starting weight and inflammation scoring. The speed at which the animals regain body weight during therapy is also monitored.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation and tissue remodelling are complex procedures involving many different cell types working as a system. Although the applicant has developed a way to use human clinical samples to replace some of the use of animals, to study gut inflammation and tissue remodelling in the pre-clinical setting still involves the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We also propose to use the high-resolution miniature endoscopy and microCT scanner to monitor colitis severity development therefore reducing the number of animal to be tested. We improve our analytical skills and reduced the number of animal per group. By changing the experimental models, we have reduced the number of mice we use.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The proposed experiments fulfil the SMARTER criteria in that they are specific with defined endpoints and measurable quantifiable readouts to avoid using excessive number of animals. We choose to use short-term, reproducible models to minimise the use of animals, pain suffering, distress or severity. The whole body weight will be measured daily to monitor the welfare of the animals and to give an indication of the efficiency of the treatments. Body weight will drop at the onset

	of inflammation and the body weight will rise when the inflammation is resolved. The experiment is usually terminated at the well-defined point. In the rare cases, when the animals become sick, a schedule 1 killing will be performed for humane reason before the planned end point. For long term therapy or pharmacological studies, if there is no clinical improvement (no body weight gain) by week 3, the mice will be killed by a schedule 1 method. We will plan a pilot study to demonstrate proof-in-principle using two or three animals per group and lower dose of substances to establish a refined protocol before going on to do more severe definitive studies. They are relevant to the important issues of inflammation and immunity in the gut and in other organs and they can be easily evaluated by analysis of the outcomes in publications and presentations.

<table>
<tr><td>Project 2</td><td>Prevention and diagnosis of infectious diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neisseria; Chlamydia; Norovirus; vaccines ; diagnostic reagents</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>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>No broadly-protective vaccines exist for serogroup B meningococcal (MenB) infection worldwide, and their absence reinforces the need for effective treatments for patients with disease, where morbidity and mortality is still between 20-50%, even with antibiotic use. There are also no vaccines for preventing gonococcal and chlamydial infection, which are both rising dramatically worldwide. To develop chlamydial vaccines, scientific knowledge is needed of its gene structure and functional biology, which are dependent on the production of new laboratory reagents and techniques. There is also an urgent need for broadly-reactive diagnostic reagents for norovirus infection, which are the major cause of community outbreaks of non-bacterial gastroenteritis.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be</td><td>These will develop new vaccine antigens for preventing bacterial meningitis/sepsis and important sexually transmitted diseases. Important</td></tr>
</table>

advanced or humans or animals could benefit from the project)?	efficacy data on anti-inflammatory sepsis treatments will inform next-stage studies in man. The project will also lead to diagnostic reagents and techniques that can be used for pathogen rapid-detection systems and for epidemiological studies of infection. Thus, our program will inform public health intervention strategies for major infectious diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	All experiments are designed to minimise the animal numbers used for obtaining statistically valid data and for ensuring a commitment to ***r***efinement, ***r***eplacement and ***r***eduction of animal usage. Over the 5 year term of the project we anticipate using ~1200 adult mice and ~25 adult rabbits for antibody production, 200 adult mice for infection studies, ~20 adult rats for antibody production and 10 for infection studies (pup delivery) and ~100 infant rats for passive antibody protection studies. High animal welfare standards will be met with environmental enrichment, good husbandry and frequent monitoring.
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 experiments are designed to minimise the animal numbers used for obtaining statistically valid data and for ensuring a commitment to ***r***efinement, ***r***eplacement and ***r***eduction of animal usage. The majority of Few adverse effects are expected, but they will be immediately ameliorated if they occur and affected animals will be humanely culled. None of the models use death as an end-point measurement.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no reliable alternative *in vitro* models for determining the potential of candidate pathogenic *Neisseria* and *Chlamydia* vaccines. The development of diagnostic immunological tests for infection also requires a wide range of antisera. For these objectives, the use of animals is essential as antibody production requires a fully functional immune system that cannot be reproduced *in vitro*. Drug efficacy trials in whole organisms are necessary, since infection

	processes are multi-factorial with many tissues and organs involved.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments are designed to minimise the animal numbers used for obtaining statistically valid data and for ensuring a commitment to ***r***efinement, ***r***eplacement and ***r***eduction of 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.	The Home Office 'Antibody Production: Principles for Protocols of Minimal Severity' (Home Office 2000) guidance will be adhered to. To minimise discomfort during collection of blood local anaesthetic will be used. Freund's adjuvant will only be considered if the other non- toxic adjuvants prove unsuccessful at stimulating adequate antibody responses. Distress will be determined using the guidelines issued by the LASA Working Party on the Control of severity of scientific procedures on laboratory animals (Wallace, J., Sanford, J., Smith, M. W. and Spencer, K. V. (1990) The Assessment and Control of the Severity of Scientific Procedures on Laboratory-Animals. Laboratory Animals 24, 97-130).

<table>
<tr><td>Project 3</td><td colspan="3">Autoimmunity in immune-privileged environments</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Multiple sclerosis, uveitis, autoimmune disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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></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">To discover the mechanisms that control persistent autoimmune inflammation in tissues which are ignored by the normal immune system. In disease the immune system attacks these tissues as if they were infected, although they are not. The project will investigate what initiates and perpetuates this state.</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">Advancing our understanding of autoimmunity in humans and animals.

The discovery of drugs that promote the resolution of chronic inflammation in the context of autoimmunity.</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">Mouse - 1300 per year

Rat - 4 per year</td></tr>
<tr><td>In the context of what you</td><td colspan="3">Animals will experience transient mild discomfort</td></tr>
</table>

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?	related to handling as part of standard breeding programmes This will apply to the majority (>80%) of animals. Animals involved in clinical experiments will experience short-lived mild discomfort in respect of injections and clinical assessment (50%). Animals undergoing general anaesthesia (10%) will experience moderate discomfort for a short period of time. Animals with clinical disease sufficient to impair normal activities (5%) will experience moderate discomfort over 2-5 days. Animals will be killed at the end of experiments
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Autoimmune disease is a complex clinical state involving many different types of immune cells. It cannot be modelled in simpler animals with more primitive immune systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are designed to progress from testing on cells through simple test on animals before testing in models of disease. Experiments are designed using group sizes that have an 80% chance of revealing a biologically meaningful effect.
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 used because it is already established that drugs can be successfully developed based on experiments carried out in these animals. They are also the best understood mammalian model in which genetic modification has been used. This offers the opportunity to refine experiments which and can reduce the number of animals that need to be tested. We use the mildest disease model of immune system disease that mimics human disease to discover new therapies.

<table>
<tr><td>Project 4</td><td colspan="3">Leucocyte migration and immunity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Leucocytes, blood vessels, tumours, viruses,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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></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>
</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">An important feature of the immune system is to generate activated leucocytes that are able to clear infections and repair damaged tissues as rapidly as possible. However, tumours are able to limit the entry of killer leucocytes and avoid being destroyed by the immune system. The aim of this project is to understand what signals pass between leucocyte and blood vessels that allow entry into infected tissues but not into tumours.</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">Persistent leucocyte infiltration is commonly associated with chronic inflammatory diseases such as rheumatoid arthritis, asthma, multiple sclerosis and cardiovascular disease (atherosclerosis) and is intimately involved in the associated tissue damage. The ability to interfere with leucocyte traffic could provide selective regimes of immunosuppressive therapy for these diseases and for other inflammatory conditions such as autoimmune diseases, hypersensitivity reactions and graft rejection. The cell adhesion molecules</td></tr>
</table>

	that regulate leucocyte recruitment from the bloodstream to sites of inflammation are targets for immunotherapy and antibodies to leucocyte adhesion molecules are currently used in the clinic. The idea of promoting immune responses to infections or tumours that can evade the immune system by manipulating immune cell homing is relatively unexplored. The results of this research will benefit academics and biotechnology industries interested in how immune responses are regulated by leucocyte trafficking and provide training for postgraduate and postdoctoral science and medical students. It also has educational benefit to undergraduate and school students in understanding the complex interplay between the immune system and cancer. There is currently a lot of interest in the use of immunotherapy to control cancers and this research will be of general interest to the public. Identification of strategies to improve the effectiveness of immunotherapies will contribute to improving the health of humans and animals by combating chronic debilitating diseases such as cancer, autoimmunity and chronic infection.
What species and approximate numbers of animals do you expect to use over what period of time?	It is estimated that approximately 5000 mice will be used during the 5 year course of this proposal.
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?	Leucocytes carrying non-toxic markers will be analysed by sampling the body fluids and tissues of mice in which immune-mediated tissue damage is measured. A typical experiment would aim to understand the role of a particular homing molecule by treating the animal with agents that inhibit the signal or by using genetically altered animals which are deficient in specific signals and measuring the effect on leucocyte migration and the extent of tissue damage. In some experiments animals will be vaccinated or treated with agents that dampen down the immune response before determining effects on leucocyte migration. The majority of protocols are simple and involve only transient

	discomfort, usually after a single injection. Some protocols will require a greater potential for discomfort, such as those involving infection with live microorganisms or tumour growth. For these 'moderate' protocols a detailed scoring system will be used to assess the welfare of the animals and humane endpoints will be used to prevent additional adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Some aspects of leucocyte migration can be studied *in vitro* such as the recruitment of leucocytes across cultured blood vessel cells. However, the 3-dimensional organisation of tissues and blood vessels cannot be mimicked in a tissue culture dish or by computer modelling. Therefore animal models need to be used for the generation of definitive data. The animal model to be used is the mouse as, over the past few decades, a large body of information has been gathered about the mouse immune system. Much of this information has shown that immune responses in mice closely parallel those in humans. Mice are well defined immunologically, allowing us to reduce the number of unknown factors in any given experiment and increasing our chances of obtaining interpretable and meaningful data. In addition, genetically altered mice expressing mutations in cell adhesion and signalling molecules known to regulate leucocyte migration are available and provide an ideal opportunity to analyse their roles in regulating the integrated function of the immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The experimental models are well established and individual experiments will be designed with the aid of appropriate statistical analyses to ensure that no more animals are used than required for statistical validity.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most	The overall architecture and distribution of lymphoid tissues is very similar between mouse and man. Importantly, many of the homing associated molecules that guide leucocyte entry into tissue are highly conserved between mouse and man and

refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	hence experimental data gained about the role of these molecules in mouse leucocytes are very likely to apply to humans and other animals. There exist in the mouse defined genetic mutants lacking expression of specific homing proteins and the potential to generate transgenic mice expressing specific homing proteins which provide an ideal opportunity to perform detailed analyses of their roles in regulating immunological function. Mice are well defined immunologically, allowing us to reduce the number of unknown factors in any given experiment and increasing our chances of obtaining interpretable and meaningful data about leucocyte homing and immunity. The procedures to be carried out during the course of this project are moderate in severity due to the impact of viral infection and tumour burden. The cumulative severity for a single mouse is minimised by the use of anaesthesia and by resting mice in their home cage between multiple procedures carried out on a single day. To minimise harm, animal welfare during the course of viral infection and tumour growth will be monitored daily using multiple parameters including appearance, behaviour and clinical signs and animals treated accordingly.

<table>
<tr><td>Project 5</td><td>Prevention of bacterial meningitis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Vaccines, bacteria, pathogenesis, meningitis</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>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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>There is still an urgent need for novel vaccines to protect individuals from bacterial meningitis. This condition affects mainly children and young adults; case fatality rates are still around 10%. However the available vaccines do not cover all serogroups of Neisseria meningitidis, and provide no protection against the commonest form of disease, MenB. Several major pharmaceutical companies and many academic groups are attempting to discover antigens for inclusion in vaccines against the meningococcus.

Currently we do not have a full appreciation of:

1) how the bacterium interacts with its host to cause bloodstream infection. By identifying the key components of the meningococcus that are required for disease, we should be able to identify targets for prophylactic immunisation or novel therapeutics.

2) the immunological basis of protective immunity. Understanding these fundamental aspects of the interaction between the pathogen and host are</td></tr>
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	essential for the design of novel approaches to prevent and effectively treat 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)?	There are estimated to be between 50,000 and 100,000 deaths each year worldwide from meningitis with a significantly larger number of children suffering from severe disability (amputation, neurological disability) following infection. Therefore there is an urgent need to develop new ways to protect vulnerable individuals from this life-threatening and debilitating condition. So far, we have some knowledge on how this important human pathogen evades our immune system, and vaccines have been introduced that are effective against some strains. However there remain no broadly effective vaccine against the most common form of disease in Europe and North America. Our work in previous licences has led to Phase I and II vaccine trails that have contributed to understanding the basis of protective immunity. Our work is undertaken in collaboration with pharmaceutical companies as this will allow the successful translation of work performed under this licence into human benefits. Therefore, further advances are expected to be gained from the licence under consideration.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of this licence, it is estimated that up to 500 infant rats, 12600 adult mice and 100 rabbits will be included 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?	Most will be killed using a schedule 1 method, with others killed by exsanguination under terminal anaesthesia. Following challenge with live bacteria, we have changed the endpoints of the experiments and instituted extensive monitoring of animals, so these procedures are now moderate severity. Breeding of genetically modified mice is likely to be mild. Other mice will be for the generation of antibodies and then schedule 1 method etc.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible, experiments are undertaken with in vitro model systems including whole blood assays, serum assays, and assays involving cell culture. However as there are no adequate models to replicate the complexity of the mammalian immune system and circulatory system, some experiments require the use of animal models of infection.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are planned to keep numbers in control groups to a minimum. Furthermore we use approaches (including signature tagged mutagenesis and the measurement of competitive indices) that have been specifically designed to reduce the number of animals used in 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.	It would be inappropriate to use non-mammalian animals for studying meningococcal pathogenesis, immunity or for the generation of antibodies as their immunological systems do not adequately reflect the human immune system. Rodents are the lowest form of mammal that have been used to study meningococcal infection, and these models have been extensively evaluated and applied for understanding pathogenesis, and for identifying vaccine candidates. Unlike adult animals, infant rats are susceptible to meningococcal challenge. This mirrors the situation in the human host in which infants are the most at risk from meningococcal disease. Rabbits are used for production of polyclonal antibodies as they are of a size capable of producing sufficient quantities of immune sera, have a relatively long life span, and are relatively easy to handle. The end point of the work is the development of bacteraemia; this occurs before Animals are monitored closely throughout experiments to see if they fulfil specific pre-defined criteria. These are in place so any animals in distress will be rapidly identified and killed by a schedule 1 method. Procedures are kept to a minimum and performed as quickly as possible and using methods to reduce any potential discomfort.

<table>
<tr><td>Project 6</td><td colspan="3">Antigen-specific immunotherapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Autoimmune, Multiple Sclerosis, T-lymphocyte, Gene, Immunotherapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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>
</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">Our laboratory is seeking ways to improve treatment of autoimmune diseases and allergies. Our novel approach is to use fragments of the molecules that are attacked by the immune system to switch off the response without the need to use potentially dangerous immunosuppressive drugs. So far this has worked in experimental models of disease and we are currently testing the approach through clinical trials in patients with multiple sclerosis. However, we understand very little about how this works.</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 work that we will do over the next 5 years will reveal much about the mechanisms involved in antigen-specific immunotherapy. This will help ensure the optimal safety and will lead to improvements of the efficacy of this type of treatment.</td></tr>
<tr><td>What species and approximate numbers of</td><td colspan="3">We will use mice that may be genetically modified in order to understand the role of specific genes in</td></tr>
</table>

animals do you expect to use over what period of time?	immunotherapy. We breed approximately 3500 mice per year and expect to use 17500 over the lifetime 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?	The most severe part of the project is to induce a disease called experimental autoimmune encephalomyelitis (EAE) in some of the mice. EAE is the closest we can get to the pathology of multiple sclerosis in experimental animals. Mice with EAE can lose weight develop a varying degree of disability including paralysis of the hind limbs. Multiple sclerosis is a devastating disease for which there is currently no safe and totally effective treatment. The EAE model has a severe level of severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We study the adaptive immune system. This is extremely complex and sophisticated in mammals. The function of the immune system is poorly reproduced in vitro and is impossible to study in invertebrates.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have created transgenic animals that allow us to use far fewer animals to generate unequivocal 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 best characterised species for investigating the mammalian immune system. While there are subtle differences between mouse and man, the major building blocks of the adaptive immune system are the same. Genetic manipulation of the mouse through transgenesis, gene knockouts and the shRNA approaches described in this project are the most sophisticated available; they will enable us to understand the mechanisms of antigen-specific immunotherapy most precisely thereby limiting the numbers of animals required. Most of the work described depends on the analysis of immune responses ‘ex vivo’ i.e. using cells isolated from lymphoid organs. This results in minimal harm. However, we are

	seeking to improve the treatment of a human disease, multiple sclerosis, and ultimately any advances in treatment will have to be tested in the most appropriate animal model for this human disease, the EAE model.

<table>
<tr><td>Project 7</td><td colspan="3">Pathogenesis and control of respiratory diseases.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Ruminant disease; respiratory disease.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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></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>
</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">To study how the microbes that cause bovine respiratory disease (BRD) interact with each other and the cattle immune system to induce disease. Also to understand how the viruses that cause malignant catarrhal fever cause disease. By doing this we will improve diagnostic tests and disease control strategies, particularly vaccination. We will also study how to improve the duration and effectiveness of the vaccines, which is currently an area of scientific need.</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 envisaged studies will generate novel and important data that will resolve mechanisms of how BRD and MCF diseases are initiated and consequently use the information to improve current diagnostic and disease control strategies. Improving veterinary vaccines using these diseases as initial targets for study is an important desirable outcome.</td></tr>
<tr><td>What species and approximate numbers of animals do you</td><td colspan="3">The project is over 5 years.</td></tr>
</table>

expect to use over what period of time?	Cattle 772 Rabbits 312 Hamsters 204. Mice 288.
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 procedures (e.g. blood sampling, dosing) are mild and cause no more than transient discomfort and no lasting harm. At the end of a set of regulated procedures animals will be inspected by the NVS or nominated veterinary surgeon and killed humanely by a Schedule 1 method. MCF**:** Moderate severity. Rabbits, hamsters and cattle react to infection by developing a febrile response on day one of the disease followed by any or all of a range of symptoms including lack of appetite, depression, discharges from the nose and eyes. This is monitored by expert staff and the NVS and animals given appropriate veterinary treatment. Failure to respond and progression from moderate to severe severity will mean animals will be humanely euthanized by a schedule 1 method. For BRD (moderate severity), cattle infected with the viruses, bacteria and mycoplasmas causing BRD develop disease manifested by inappetance, coughing, respiratory distress and occasionally diarrhoea. Animals will be monitored, treated and subjected to humane euthanasia by schedule 1 method as described above for MCF.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All the envisaged animal experiments are necessary to achieve the objectives of the project and cannot currently be replaced by any other system or approach. This is because the bulk of the animal work is either to study pathogenesis (disease induction) where interactions between different pathogens and pathogen and host can only be studied currently *in vivo*, due to the complex interactions of physiological, immunological and pathological responses; or vaccine studies that require assessment of efficacy in vaccinated and challenged

	animals. In vitro work will be used, where appropriate, to look at details of microbial interactions with cells that will inform animal disease studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In consultation with our statistician, we will use known variation and predicted responses in power calculations to calculate the minimum level of replication required to provide adequate statistical power for each experiment. When appropriate, we will use covariates and crossover designs to minimise residual variation and reduce the number of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Cattle are naturally susceptible to BRD and MCF. They will be the principal species for vaccine and disease studies as they are economically important and the disease(s) are a major welfare problem in this species. Rabbits and hamsters have been identified as providing an accurate model for MCF and can be used to determine mechanisms of disease that will inform cattle experiments. Mice are a good model for studying vaccine immune responses as there is a large reagent and technique base for studying host responses in them. All these are the most appropriate animal species for the envisaged studies. Furthermore, for each of the animal species and pathogen (causing BRD or MCF), infection regimes have been developed by other laboratories as well as our own to maximise the likelihood of obtaining important data for our understanding of disease progression and control. We will follow the most refined protocol for each pathogen and host infection experiment. All animals will be maintained to the highest standard of husbandry and care in facilities designed to provide the best possible welfare standards. Procedures will be performed only by suitably competent operatives using appropriate handling facilities to minimise stress on animals. In all cases where there are alternatives, we will utilise the procedure that imposes the least harm to an individual animal.

<table>
<tr><td>Project 8</td><td>Role of Innate Immunity in diseases of the CNS</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunity, Brain, Complement, Dementia, Inflammation</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>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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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Complement comprises a network of proteins in plasma and on cells that collaborate to defend against infection. Complement dysfunction can contribute to many diseases, particularly diseases of the nervous system such as multiple sclerosis and dementia. The primary objective of the project is to develop a comprehensive understanding of the role of complement and related immune molecules in a group of neurological diseases linked by the presence of inflammation. A secondary objective is to inform the development of better diagnosis and treatments for these diseases, an enormous unmet need.</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 potential benefits include better tests for disease classification and staging and better drugs for treating inflammatory aspects of these diseases.</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	Mice (approximately 900) and rats (approximately 300) over the five years of the Programme.
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 each of the disease models proposed in the project, animals are expected to develop a neurological disease; in the case of protocols 1 and 2 the anticipated disease is a relatively acute onset of paralysis akin to human multiple sclerosis or myasthenia while disease in protocol 3 is a slowly developing neurodegeneration resembling Alzheimer's disease in man. Animals will be carefully monitored and when the disease severity reaches a moderate level of severity as assessed clinically and by measuring weight loss, the animals will be humanely sacrificed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no alternative to the use of animal models in order to unravel mechanisms by which the immune system contributes to the modelled human diseases and to explore which drugs might slow or reverse disease. Animal experiments are supported by a large body of test-tube and cell culture experiments that answer some of the questions and help in the design of animal experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary non-animal experiments can help in the choice of agent, dose of agent and duration of treatment in the animal experiment, reducing the need for initial dose-finding or effectiveness animal tests. Statistical help in study design ensures that enough but no more animals are used to arrive at an answer to the question set.
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	Tests on the immune system to be of relevance to man require a species that has an immune system similar to man. Rodents are ideally suited in that they have comparable innate immune systems and there are many reagents available to help us measure changes in their immune systems with disease. Harm will be minimised by excellent experimental technique throughout, close

minimise welfare costs (harms) to the animals.	monitoring of animals throughout, prompt termination when set severity thresholds are reached, excellent husbandry to ensure easy access to food, water and other environmental supports, and always an attitude that harm must be kept to the minimum necessary for the experiment.

<table>
<tr><td>Project 9</td><td>Pattern Recognition Receptors in Bone Remodelling</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bone, immunology, receptor</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>Yes</td><td>X</td></tr>
<tr><td>Translational and applied research</td><td><s>Yes</s></td><td>No</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>Greater understanding of the underlying mechanisms that regulate bone formation and loss. Formation of new bone (remodelling) occurs throughout life and is the result of activities of cells that either remove bone or make new bone. This balance is critical for health.</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>Basic biology of bone loss and formation. Greater knowledge will aid our understanding of the pathology of multiple human diseases in which bone formation is affected and identification of new points of therapeutic intervention. Understanding of a novel murine model (SCARA5) of altered bone re-modelling.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice

We anticipate that ~ 8500 mice in total will be maintained over 5 years</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td>Our use of mice is predominantly to provide material for in vitro studies. Our in vivo studies are made only when they cannot be modelled in vitro. Animals that undergo surgery will experience</td></tr>
</table>

level of severity? What will happen to the animals at the end?	transient pain, which will be controlled by appropriate use of analgesics (protocol 3). Mice that undergo bone marrow transplantation (protocol 3) may suffer transient diarrhoea as a result of irradiation. Animals that receive inflammatory/ immunological challenge may display transient sickness behaviour (protocols 3 and 4). At endpoint all experimental animals will be killed by schedule 1 or terminally anaesthetised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We undertake the majority of our experimentation using tissue culture models. Provision of tissues and cells for such investigations is the major fate of mice. Unfortunately immortalised cell lines currently available do not replicate all the biological and genetic properties of *ex-vivo* primary cells. However, in order to achieve our objectives there is a clear need to study intact body systems because complex systems such as the immune system and the organised skeleton cannot currently be replicated *in vitro*. Whenever possible we initially undertake *in vitro* investigations the data from which is used to design and guide *in vivo* studies. Because we study bone formation, use of evolutionary more primitive species e.g. invertebrates is not possible
2. Reduction Explain how you will assure the use of minimum numbers of animals	A minimum colony size sufficient to generate animals for our investigations will be maintained for each strain. With advice from our NACWO we monitor animal numbers and adjust breeding programmes accordingly. We carefully consider minimal group sizes, number of groups to be studied, use of one or both sexes of animals as appropriate and optimisation of protocols. Small-scale pilot studies are always conducted to see if larger scale studies are warranted. We are committed to ensuring reduction of animal numbers through appropriate experimental design and looking for alternative approaches and using statistical analysis packages that we only use the minimum required to generate statistically

	significant data.
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 evolutionarily the lowest animal group that have the skeletal form, complex immune system and genetic modifications that will allow us to generate significant data relevant to human disease. Each procedure will be initially performed on a minimum number of mice to establish and define the shortest time required to generate significant data. Cumulative suffering is minimised by carefully designing experiments to prevent individual animals being exposed to multiple protocols unless there is no alternative. We have a continuous dialogue with our collaborators and others to ensure we are aware of and employ the most up to date approaches.

<table>
<tr><td>Project 10</td><td>Strategies to promote foreign transplant survival</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Transplantation, GVHD, T cells, iNKT cells, Treg</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></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>The success of transplantation as a life saving procedure depends on our ability to prevent rejection or to prevent the graft from attacking the host (GVHD) following bone-marrow transplantation. Although current immunosuppressive drugs increase transplant/patient survival they are not 100% effective at preventing rejection/GVHD, are often toxic and cause a dramatic increase in the chance of transplant patients getting cancer or life-threatening infections. Therefore, it is clear that new anti-rejection/GVHD therapies are needed that work better, do not need to be taken for life, are not toxic and do not suppress all immune responses but only those that target the transplant.

With this in mind, the objectives of this project are:-

(a) To determine the potential of different immunosuppressive cells for controlling T cell-mediated transplant rejection/GVHD.

(b) To define some of the cellular and molecular</td></tr>
</table>

	mechanisms used by immunosuppressive cells to suppress transplant rejection/GVHD. (c) To develop therapeutic strategies that target T cell responses to transplants or bone-marrow transplant recipients.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this work are clear in that the discovery of any therapy that will consistently prevent transplant rejection without side-effects would be a significant advance in human health and disease. More specifically:- Objective 1 - This part of the work will provide information on what immune cells are able to suppress rejection. This information will advance our ability to use these cells as a potential cellular therapy to combat transplant rejection and GVHD. Objective 2 - This part of the work will advance scientific understanding of how such immunosuppressive cells function in the normal immune system as well as in the response to a transplant. In addition, we expect this work to reveal new molecular/cellular pathways that could be targeted as part of anti-rejection/GVHD therapy. Objective 3 - New molecular/cellular interactions used by immunosuppressive cells (identified in objective b) will be targeted to determine whether this prevents transplant rejection. This information may be used by us and other researchers to develop new therapeutic strategies that may prevent transplant rejection/GVHD in human transplant patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that a total of 11,000 mice will be used in the 5 years covered by this project license. Although the license pertains to transplantation approximately 25% of mice will be used for breeding only and a further 50% of mice will not receive a surgical procedure.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	All transplants in the mouse will be performed in a dedicated operating suite by skilled personal license holders according to strict Home Office, Institutional and laboratory guidelines and kept under constant review by an institutional review

level of severity? What will happen to the animals at the end?	panel and institutional veterinarians. Anesthetic, analgesia and post-operative recovery protocols have been developed in close consultation with the institutional veterinary service and represent current best-practice for the minimization of distress. Recovery from surgery is closely monitored and importantly animals return to normal patterns of behavior (movement, feeding, drinking) within twelve hours of surgery. Monitoring of transplant outcome requires a simple visual inspection of the transplant (skin grafts), finger-tip evaluation of transplant function by palpation (heart grafts) or simple blood test (islet grafts). In order to assess whether islet transplants are functional, recipient mice will need to be made diabetic by the administration of an islet-toxic drug called streptozocin (Stz). This will cause diabetes that may result in weight loss, increased urination and therefore dehydration. The effect of Stz on health will be transient as following islet transplantation normoglycemia will be restored. The health of mice will be monitored daily throughout this period and any mice found to exhibit a significant deterioration in health will be humanely killed. The likely level of severity is Moderate. At the end of experiments (on average within 50 days of the first procedure) the animals will be humanely killed by a schedule 1 method or under terminal anaesthesia. Transplants and immune cells will be recovered from such animals for histological, functional and genetic analysis.
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 transplanted tissue is complex and thus far has not been accurately modelled in vitro due to the importance of the local micro-environment and the trafficking of donor cells to the recipient as well as the homing of recipient immune cells to the transplanted organ. With this in mind, the use of animal models of transplantation is the only way to accurately assess the immune response to transplants in a way that is translatable to clinical transplantation. That said we

	continuously evaluate new in vitro methods for investigating immune responses to transplanted organs/tissues/cells and use in vitro methods wherever possible. For example, we have developed methodologies to generate cellular therapies in vitro, and use in vitro techniques such as MLRs and IFN-□ ELISPOT to explore mechanisms of immune regulation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To determine sample size for the assessment of non-parametric graft survival a Wilcoxon-Mann-Whitney t-test (two groups) was used where the parental distribution was set to asymptotic relative efficiency (ARE). The analysis revealed that a maximum of 6 animals per group would be needed to yield significant results ($p<0.5$; 80% power) which is consistent with our previous experience. For the comparison of immune responses to an allograft power-analysis revealed that 3-5 mice per group would be suitable to yield significant ($p<0.05$; 80% power) results. The power-analysis used was a t-test for 2 independent means (2 groups) with common variance. GPower 3.1 statistics software for all power-analyses.
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 studies will be carried out in mice where many well-characterised and genetically modified strains are available. The models of heart, skin, islet and bone-marrow transplantation are the most extensively characterised and used models of these sorts of transplant in the mouse. These models have been shown to accurately reflect immune responses to human tissue and cell transplants and to result in the maximal acquisition of information whilst causing the minimum of suffering to mice. For example, most transplants will not replace the animals own organ i.e. in the heart transplant model mice receive a heart transplant but the animals own heart is left in place meaning that the graft is not life-supporting and the rejection of the graft causes

	no suffering to the animal. Similarly, skin transplant rejection involves shrinkage of the graft and would healing that has no impact on the health of mice. The islet transplant model requires mice to be made diabetic (by Stz) which may cause transient weight loss, overproduction of urine and dehydration. Such welfare cost to diabetic mice will be minimised by careful dosing of STz (to reduce side-effects), increased cage changing (to prevent build up of wet bedding), careful monitoring of animal health and islet transplants performed as soon as possible after diabetes induction (normally 5 days to ensure that Stz is cleared and does not kill the islets in the transplant). In addition, the majority of therapeutic interventions are brief, induce little in the way of side-effects and much of the analyses of immune cells and responses will take place in vitro after the in vivo experiment has been humanely ended.

Project 11	**Understanding the role of FcyRIIB in health and disease**	
Key Words (max. 5 words)	Antibody, immune-regulation, infection, autoimmunity, kidney-injury	
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)	We are interested in antibodies, which are soluble immune components that circulate in the blood. Antibodies are important for defence against many infections and for the protective effects of all effective vaccines. They are produced by immune cells called B cells and work by binding to pathogens and engaging other parts of the immune system via receptors called Fc receptors. Unfortunately, antibodies can also play a role in diseases, particularly autoimmune diseases (like lupus and inflammatory bowel disease) and in the rejection of transplanted organs by engaging the immune system against our own tissues or a transplant. They may also contribute to inflammation during tissue damage, including acute kidney injury (AKI).	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	By improving our understanding of how antibodies cause damage to transplanted kidneys, we will identify therapeutic targets to both prevent and alleviate this problem. This will be of help to patients with a transplant, and to those with kidney failure awaiting transplantation. There are currently around	

project)?	6000 such individuals in the UK and 30% are sensitised (Ie. they have detectable donor-specific antibodies). Identifying factors which allow us to predict the pathogenic potential of alloantibodies will allow more patients to be transplanted and better allograft survival, avoiding the need for dialysis. This has significant economic implications since dialysis accounts for 2% of total NHS spending. It has been estimated that kidney transplantation saves approximately £20,000 per year per transplant for the lifetime of the graft. The avoidance of dialysis also has societal benefits, since many patients receiving dialysis are unable to work due to the time constraints imposed by the requirement for thrice weekly dialysis sessions. Transplantation restores patient independence and facilitates return to work. Our work will also examine how and when receptors for antibodies (particularly the receptor called FcyRIIB) can control their adverse and beneficial effects. This could help in optimising vaccination strategies, in treating autoimmune disease and diseases in which there is inflammation in the kidneys, including AKI. AKI caused by ATN affects 10% of all hospital inpatients and up to 40% of those in intensive care, with patients sometimes requiring temporary haemodialysis. This incurs a significant cost (150 per session, costing an estimated £10 million per year in the UK). Therapies which could reduce the severity of AKI would have significant economic benefits.
What species and approximate numbers of animals do you expect to use over what period of time?	Species- Mouse. 6320 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?	Many animals will simply undergo an immunisation — this is just like receiving a vaccine, and then immune responses will be assessed by taking blood samples. We will also use some disease models, a model of lupus where mice get inflammation in the kidney (this can happen spontaneously or be induced by an injection), one where a substance is added to drinking water (which causes inflammation of the intestine) and, another in which a urinary tract infection is introduced via a catheter. The mice are then carefully monitored, blood, urine, and faecal samples may be collected. We may also perform imaging studies using special microscopes or scanners, although these will

	generally be performed under terminal anaesthesia. Adverse effects vary according to protocol. For immunisation, the animal may experience mild discomfort at the site of injection. They will be carefully monitored and any excess inflammation will be managed with topical reagents according to the advice of the vet. In the disease models, we will monitor regularly for weight loss, proteinuria, or diarrhoea and mice will be promptly euthanized if severity limits are approached. All animals will be humanely killed at the end of their breeding lifespan or at the end of experiments and tissues will be further processed for detailed immunoloqy investigations.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will carry out many parts of our research using cells in culture (in vitro) to minimise animal use. We have obtained ethical permission to use primary human cells for some studies. However, although these approaches can provide some useful information, they cannot accurately model a physiological immune response, which is complex and requires a coordinated action from many different immune cell types. This response takes place within specific tissues, and is influenced by these differing environments. The complex interactions between immune cells, the tissue-specific environment and a systemic immune response cannot be recapitulated using in vitro methods hence the need for in vivo models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When designing the experiments statistical analyses ensure the use of minimum number of mice per group. We have obtained mouse strains that have fluorescent immune cells that we can image, something that is difficult to achieve using labelling techniques. This will limit the number of mice required for these studies. In addition, numerous cells can be imaged in a single field, generating multiple (10-50) data-points per experimental animal, which will also limit the overall number of mice required. To avoid breeding new knockout strains, we will use

	bone marrow chimeras. This allows reconstitution of mice in such a way as to investigate a specific immune cell type and avoids the need to generate new mouse models or perform complex crosses which require many mice. We will provide mice generated by our breeding programme to a number of other researchers avoiding other groups breeding the same strain.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will be using mice that are genetically deficient in antibody receptors and immune regulatory molecules. This allows us to examine antibody effects accurately. This maximises the quality of experimental data generated, thus minimising the numbers of animal required to test hypotheses. In order to carry out in vivo imaging studies, we have rederived fluorescent reporter mice made by expert imaging laboratories. These provide the best possible way of visualising specific immune cells. This limits the time required for imaging and optimised the amount of data which can be generated from a single experiment. All animals undergoing a procedure will be carefully monitored for signs of distress thereafter, and treated appropriately or euthanized to minimise any suffering.

<table>
<tr><td>Project 12</td><td colspan="3">Studies of invasive fungal and bacterial infection in immunocompromised murine hosts</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Bacteria, fungi, immunocmpromise, transplant</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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></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>
</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">The overall scientific objective of the project is to determine the reasons for which opportunistic bacteria and fungi are able to cause infections in patients who have received organ transplantation. This will enable identification of the primary deficiencies in patient immunity to infection that are affected by transplant immunosuppressive drugs used to stop organ rejection by the body’s immune system, and which immune cells are primarily affected. The altered immune response may also lead to the emergence of bacterial or fungal behaviours that enable infection to occur when host immune responses are suppressed. The development of new animal models of solid organ transplant infection will further enable the identification of the functional basis for any such factors. This will enable the development of novel diagnostic tests to identify at risk patient groups and enable the development of novel treatment to boost</td></tr>
</table>

	the immune system response when infection occurs
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 seeks to closely model the pathogensis of microbial infections in transplant patients. This will enable better understanding of the pathological basis for disease. This will enable the direct development of novel therapeutic strategies that seek to optimise the immune response to infection. We aim to translate these findings into clinical interventions as a direct consequence.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 5000 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 mice will receive immunosppressive drugs and then be infected with either bacteria or fungi. This will lead to establishment of infections, which may cause the animals to become distressed. The level of severity is categorised as moderate. After infection animals may also undergo administration of pharmacological agents and bioimaging, which may cause the animals to become distressed. The level of severity is categorised as moderate. At the end of the experiments the animals will be humanely culled. Suffering will be minimised as all infections will be undertaken under general anaesthesia and in addition 20% weight loss has been established as a surrogate marker for death previously (and which point mice will be humanely culled).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Microbial infections in transplant patients are complex and involve a number of hoist and pathogen factors that cannot be modelled using simple cell-based assays. There are complex interactions that occur at a whole organism and inter-organ level that need to be understood in order to develop better diagnostic and therapeutic approaches to the life threatening infections.

2. Reduction Explain how you will assure the use of minimum numbers of animals	In all instances the minimum number of animals will be used to enable statistical significance. In addition we are developing novel imaging-based models of infection that will enable further reduction of the number of animals used by allowing longitudinal studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Murine models of infection offer several distinct advantages for studying invasive fungal disease, including ease of use, reproducibility and availability of immunosuppression regimes which mimic host factors of human disease. Additionally murine models offer the opportunity for a thorough investigation of immune responses and are amenable to novel diagnostics, which falls beyond the scope of this application but will become important in our future research and will facilitate standardisation across the present research community within the context of important novel findings. Suffering will be minimised as all infections will be undertaken under anaesthesia and in addition 20% weight loss has been established as a surrogate marker for death previously (and which point mice will be humanely culled).

<table>
<tr><td>Project 13</td><td>Host and parasite genes involved in rodent malaria</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Infectious disease, malaria, parasite-host interactions, Plasmodium genetics</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></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>Malaria is one of the most important infectious diseases affecting humans. We still lack an effective vaccine, we urgently require new drugs and we need a much better understanding of how infection leads to severe 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>This project aims to gain a better understanding of parasite biology and the interactions between parasite and host in rodent models of malaria. We want to develop and apply improved genetic tools in the parasite to make the identification of targets for intervention much easier. We also want to use rodent models to understand the functions of host genes that are associated with the development of severe malarial disease in humans, which we expect will lead to better interventions and improved clinical outcomes.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Over the next 5 years we anticipate using an average of up to 5000 mice and 100 rats per year.</td></tr>
</table>

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 majority of mice (~80%) will be used for breeding or in mild protocols, in which they will not suffer adverse effects. The remainder will suffer at least some adverse effects from being infected with malaria parasites. These will mostly range from mild (some subdued behaviour, ~10% of all mice), to moderate (further reduced responsiveness and activity caused by anaemia, ~10% of all mice), and will usually not be allowed to persist for more than 48 h. A small number of mice (~1.5% of all mice based on past experience) are likely to suffer more persistent moderate effects or severe effects. This latter group may develop neurological symptoms of disease, which could include effects on coordination, movement and balance. In a very few cases symptoms in the mice may progress rapidly to coma and death, but it is important that these symptoms are studied in order to gain as much benefit from the research study as possible. All adverse effects are monitored and dealt with appropriately. All rats will be used in mild protocols only. 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	We have a programme that is developing and improving *in vitro* cell culture systems for rodent malaria parasites, which we use whenever possible. We also have *in vitro* cultured human malaria parasites in our laboratory, which we use as much as possible to replace animals and ensure the relevance of our animal research for understanding human disease. However, malaria parasites of rodents cannot be cultured in erythrocytes *in vitro*, and malaria parasites of humans are not experimentally usable for *in vitro* or *in vivo* studies at many stages of their life cycle. In addition, mechanisms of infection and disease that involve complex interactions between pathogens and hosts, such as immunity, cannot be modelled *in vitro*. For these reasons, we use rodents because they offer the best-characterised

	and least severe whole-animal model for *Plasmodium* infections.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To collect as many data points as possible while also reducing the numbers of animals we will need to use we will be using non-invasive imaging techniques that will allow us to monitor the same animal at different time points of an infection. We have also developed genetic screening technologies that allow us to query many parasite genes in a single infected mouse, greatly reducing the numbers of mice needed.
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 seek to minimise adverse effects of infection by choosing the parasite-host system with the least severe adverse effects but which still provides a robust answer to a particular important question. We will be using mice because they are the physiologically most defined and least sentient whole animal model of malaria. For different scientific questions we use a range of protocols with different severity limits, monitoring regimes and different carefully defined endpoints to ensure welfare costs are minimised.

<table>
<tr><td>Project 14</td><td colspan="3">The regulation of innate immunity by the microbiota</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Innate immunity, infection, microbiota, vaccine</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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>
</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">Humans are permanently colonized by a vast number of microbial organisms, with each of us thought to be home to approximately 100 trillion bacteria (commensal microbiota). We are therefore a “superorganism” composed of microbial and human cells. The importance of these microorganisms for our health has been reinforced by an explosion of studies showing a correlation between microbiota disruption, especially by the use of antibiotics early in life, and the increased risk of developing a variety of diseases and conditions. Microbiota disruption has been linked to cancer, asthma, arthritis, and reduced ability to fight infection. With all of these conditions it is thought disruption of the normal interactions between the microbiota and the immune system drive their development. Currently, however, the language of communication between the immune system and microbiota is poorly understood, and therefore the theme of my research is: to understand the mechanistic basis for the communication between the immune system and microbiota and how disruption of the microbiota affects our health. I</td></tr>
</table>

	have a specific interest in understanding how colonizing microbes program the function of certain types of white blood cells (called neutrophils, macrophages and dendritic cells). These key components of the immune system are found throughout our body and form the body's first line of defense against infection. Using animal models and techniques to analyze the functioning of these cells, I will focus on how, and when, colonizing microbes program these cells throughout our bodies and how this promotes responses to infection and vaccination. Furthermore, I will determine how disruption the microbiota, by the antibiotics we are commonly prescribed, cause long-term changes to own 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)?	This research aims to understand how the microbes that permanently colonize us promote immune function and how disruption of these bacterial communities causes immune dysfunction and dampens host immunity to infection. We cannot change our genetic makeup, but it might be possible to change our microbiota. If we know what microbes are important for our health, and when they are important, we may be able to re-colonize our bodies with these organisms to improve responses to vaccines and protect against infection. In the long-term, I anticipate that harnessing this understanding of host-microbiota interactions could help to protect against infection, autoimmunity and cancers.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 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?	The vast majority of models of bacterial infection and vaccination used in this work will result very few adverse effects and are well established. However, depending on the bacterial species, route of infection, and dose, some mice may develop mild to moderate symptoms of systemic infection. Animals will be assessed for these symptoms and will be humanely killed if they display 20% weight loss, and/or a combination of other symptoms (e.g. reduced activity, hunched posture or ruffled fur).
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This proposal aims to understand how our indigenous bacteria educate the immune system and how this influences host defenses to infection. This can be achieved is by using *in vivo* models, as *in vitro* systems cannot capture the complex workings of the immune system that during infection. Central to host resistance to infection is communication between immune cell types which is tightly coordinated both spatially and temporally, involving cellular migration and occurring both within, and between, different tissues. Currently, this cannot be recapitulated *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This proposal outlines the use of established models of colonization and infection that require minimal optimization thus reducing the required number of animals. Using established statistical methods, (the resource equation, http://www.nc3rs.org.uk and http://www.3rs-reduction.co.uk), I have determined the minimal number of animals required to provide biologically meaningful data while minimizing animal usage. In previous work, and in future work, animal experiments guide later *in vitro* work to model the effects of colonizing bacteria on innate cells. By using cell lines we will minimize animal usage. Furthermore, I will collaborate with other groups where possible to isolate cells from tissues they are not using and I am interested in, in order to reduce animal usage. Animal breeding strategies that minimize animal surplus will be employed throughout.
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 model of choice for understanding host-microbial interactions. This is because of the wealth of immunological tools available and experimental protocols that have already been vigorously evaluated and established. Mice are genetically tractable, enabling specific host related features to be stably controlled, or knocked out, in order to determine the basis for a particular immunological response. The models of bacterial infection used in this work recapitulate human infection accurately and thus provide extremely valuable data that is directly applicable to human disease. Non-mammalian animals are unsuited for use this work as the required immunological systems and tissues required to provide this accurate data regarding human infection do not exist in lower species.

Project 15	**Mucosal and parenteral vaccines for tuberculosis**	
Key Words (max. 5 words)	Tuberculosis, local immunity, vaccines	
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)	Tuberculosis (TB) still kills more than 1 1/2 million individuals each year and the problem is getting worse because drug resistant organisms are becoming increasingly common and because HIV infection increases susceptibility to tuberculosis. The only licensed vaccine, BCG, does not give complete protection and its efficacy varies greatly in different parts of the world. There is therefore a need for a better vaccine. Giving a vaccine by injection induces immunity in lymphoid organs (systemic immunity) and can also sometimes protect against TB but we have shown that giving TB vaccines directly to the lungs is often very effective because this induces local lung immunity. We have also shown that giving a vaccine by both routes simultaneously can increase efficacy even more. However before this strategy could be applied in humans we need to understand better how local immunity induced by immunising the lungs, and systemic immunity induced by injection, work. To do this we will imnunise with several different TB	

	vaccines and study immune responses to them, in order to identify common features of protective local or systemic immunity. Another difficulty is that we do not understand why immunising simultaneously with vaccines by different routes only sometimes gives additive protection and we will study immune responses to vaccines given singly or in pairs in order to elucidate this. Finally, we have shown that exposure to harmless bacteria can alter immune responses to a vaccine, which may be the reason that the protective efficacy of BCG varies in different parts of the world. We will work out how the harmless bacteria alter the immune response to vaccines and design vaccines that can overcome this effect.
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 more effective TB vaccine would save lives and help to prevent the spread of highly dangerous, drug resistant TB organisms. A TB vaccine that worked equally well in different environments would be highly desirable. Minimising clinic visits is important in the developing world so that a vaccine administered simultaneously, but only once, by two different routes, would be advantageous. Vaccines that are effective against human tuberculosis are very likely to work against bovine TB as weLl and bovine TB is a considerable problem in the UK, costing the farming industry and government many millions of oounds annually.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5,000 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most animals will be vaccinated by injection or intranasally, which requires light and short general anaesthesia. Some animals will be exposed to live TB and become infected. Immunisation has few side effects. Very rarely injection can cause local inflammation and intranasal immunisation can cause breathing difficulties. Animals infected with TB will be killed before the disease causes symptoms. All animals are killed at the end of the experiments. The level of severity is moderate.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is only possible to test whether a vaccine protects against tuberculosis in a living animal. Furthermore since we do not know exactly what sort of immune response is protective, it is essential to infect vaccinated animals with live TB and measure how well they are protected.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before carrying out experiments we calculate the smallest number of animals needed to obtain a statistically significant difference between experimental and control groups of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use mice because the immune system of mice has been very thoroughly studied and is very similar to that of humans. Mice can be infected with human TB and make immune responses to it, that are similar to those of humans. We will not immunise animals more than once unless this is necessary to obtain protective immune responses. Vaccines, other biological agents and drugs will be used a the lowest effective dose to minimise side effects. Animals infected with TB or other live organisms will be very carefully monitored for symptoms of disease and treated or killed if symptoms persist.

<table>
<tr><td>Project 16</td><td>Studies of antigen presenting cells in vivo</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Dendritic cells; 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 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>
</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 fundamental objective is to understand how the immune system “decides” how to react to antigen challenge.

The detailed objectives can be summarised as:

1) Which signals and pathways activate dendritic cells (DC) and how are they integrated?

2) Do all signals and pathways lead to the generation of “effector” DC with similar properties?

3) How do different DC subsets develop, what are their properties and do they respond to distinct activation signals?

4) What are the consequences of differential DC activation and DC heterogeneity for adaptive immunity and tolerance?

5) How can DC activation be manipulated to control the adaptive immune system?</td></tr>
</table>

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Cell-mediated immunity holds promise in diseases such as cancer, HIV infection and malaria where antibody-based immunotherapies have failed to deliver clinical benefit. Cell-mediated immunity to cancer is, effectively, a T cell response. The success of cancer immunotherapy, therefore, depends on our ability to prime T cells specific for tumour antigens and to steer their differentiation into effector cells capable of tumour destruction. Priming and directing T cell responses is the principal function of dendritic cells (DC), the major class of antigen-presenting cells (APC) in the body. Despite appearing as a basic research programme, this work has the potential to lead to design of better vaccines and immunotherapies for both infectious disease and cancer and to the development of immune deactivation strategies for autoimmune disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of a five year study we anticipate that we will to require up to 100,000 mice and 100 rats to undertake a project of this scope.
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 bred and used under this Licence are expected to have a lifetime experience equivalent to that of their wild-type background strains. Genetically altered mice are also expected to develop the same range and incidence of known strain-specific health conditions as wild-type individuals. For example, wild-type C57BL/6 mice have reported incidences of ophthalmic abnormalities such as microphthalmia of between 4.4%-10% and are prone to hydrocephalus and dermatitis. We expect most of the genetically altered strains used in this study to exhibit similar pre-weaning losses and display rates of adult mortality similar those of equivalent wild-type mice. We will monitor continuously for any significant increases in these rates. However, approximately 25% of mice used under this Licence will be those that may present phenotypes with the potential to exceed the mild

	severity classification. These will include such genetically altered mice as those with immunodeficiencies, a predisposition for autoimmunity or for tumour development, and those wild-type mice where such conditions are induced experimentally. Throughout this Project Licence the FELASA and NCRI guidelines, will be used to define severity categories objectively. Any individual mouse will typically undergo only a very limited number of the optional steps available and it is not anticipated that cumulative adverse effects will result from any combination of such steps. However, as it is not possible to fully predict the nature or severity of all potential adverse reactions for all types of mice undergoing novel combinations of procedures there will be careful monitoring for possible side effects. For animals exhibiting any unexpected clinical signs, such as a 20% weight loss or piloerection and an intermittent hunched posture for 24hrs the humane endpoint will be deemed to have been reached and the animal will be culled, otherwise at the end of any protocol all animals will be humanely killed. The work may eventually involve experimental models of autoimmunity such as EAE that could lead to the development of severe symptoms such as temporary limb paralysis. This is expected to be transient in the model employed, but if it persists for more than 24hrs the animal will be culled. This will involve less than 1% of the animals used in the project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Research into the cellular and molecular interactions that determine the outcome of antigen challenge requires an intact immune system. Therefore, by definition, such research cannot be carried out in vitro. The development and function of the immune system involves many different cell types interacting in a dynamic three-dimensional environment. For example, the progression of an

	infection within a whole organism involves changes of antigen expression and presentation that evolve with both time and spacial distribution. Similarly, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding cells, governed by multiple signals originating from both their immediate neighbours and from distant tissues. These factors combined with the involvement of multiple host cell-types and the clonal expansion and migration of effector cells mean such research cannot be carried out in tissue culture alone and can only be addressed by the use of animals. The mouse is one of the model organisms that most closely resemble humans. The human and mouse genomes are approximately the same size, and display an identical number of genes, which are functionally conserved. Further, mice have genes not represented in other animal model organisms (e.g. *Caenorhabditis elegans* i.e. nematode worm*,* and *Drosophila melanogaster* i.e. fruit fly) such as those involved in the adaptive immunity. Mice can be genetically altered, there is extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will collect as much evidence as possible from current literature, and through the analysis of available data. We will also perform studies in vitro using established cancer cell lines and mouse primary non-transformed cells. These studies will precede and guide the generation of relevant transgenic mouse models. We will minimise the number of animals by mostly using inbred mouse strains, and by housing them under identical conditions to limit variability. We will avoid overbreeding, and lines under sporadic use will be maintained at low levels, and frozen whenever practicable, and/or maintained in

	collaboration with other licences to minimise redundant breeding. The proposed experimental designs and methods of analysis will be discussed with members of the laboratory, and those of our collaborators, and we will seek additional advice from the statisticians employed by our Institute. We will perform pilot experiments for comparing genotypes using small numbers of animals per group. If some effects are worth investigating further we may perform larger cohort studies to determine if the observed difference is statistically significant. The size of the cohort will depend on the observations made from the pilot studies, and will be determined using power calculations. We aim to use the minimum number of mice per group that will be informative.
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 use of inbred and fully backcrossed mice in the field of immunology not only reduces intra-group experimental variability but also eliminates MHC incompatibility when cell transfers are carried out between various knockout, transgenic and wild-type strains. Without such a defined genetic background nearly all of these experiments would be impossible. In addition, we use specific genetically-modified animals to understand the molecular events and steps involved in the immune activation process or as a way to direct immune responses against defined model antigens, thereby making analysis and quantitation of immunological effects easier. The categories of genetically modified mice necessary for achieving the objectives of the project include; i) Strains expressing transgenes which play a role in, or with expression targeted to cells involved in, immune function and regulation. ii) Strains with the absence of, or modifications to, genes involved in both the innate and adaptive immune system, examples include pattern recognition receptors, components of

	signalling pathways, lymphocyte surface markers and receptors. iii) Strains expressing DNA recombinases and/or reporter genes of such recombination. iv) Strains expressing oncogenic transgenes that increase the incidence of spontaneous tumours. v) Strains developing (or with an increased tendency to develop) spontaneous autoimmune or inflammatory conditions due to transgenic modification or mutation. vi) Crosses of such strains. Whenever possible we will generate transgenic mice in which mutations are induced specifically and conditionally e.g. using Cre-LoxP conditional alleles, where mice should not display a phenotype until the mutation in the candidate gene is induced. Where the immune status of the animals might compromise health, they will be maintained in isolators or IVCs (individually ventilated cages) under barrier environment, to avoid infections. In our experiments we will set clear humane endpoints and will for each and every experiment, as part of good laboratory practice, write an experimental protocol, which will include details of possible adverse effects. These experimental protocols will be provided to all the staff involved in the experiment. In addition, when considering which route of administration of substances to employ, we will strive to use the least invasive route whilst maintaining direct control of dose. The choice of route to administer a substance or cells will be such as to achieve “best practice”, i.e. to minimize or avoid adverse effects, reduce the number of animals used, and maximize the quality and applicability of results. For that reason we propose in this project licence a variety of routes of administration of substances and cells to achieve the scientific objectives. Although in the majority of cases we will primarily use standard routes of administration such as intravenous or intraperitoneal injections, the active concentration, volume, stability, and toxicity of a particular

	substance or cells may require administration through a non-standard route such as injection intratumourally or peritumourally. For all procedures coded (AB) or (AC), general or local anaesthesia as appropriate will be induced and maintained using agents and routes of administration suitable for the species and the nature and duration of the procedure.

<table>
<tr><td>Project 17</td><td colspan="3">Basic and applied aspects of T cell immunity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Adaptive immune system, T cell receptor, tumour, antigen</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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>~~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>
</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">The immune system comprises a complex network of tissues, cells and molecules that efficiently recognise, respond and eliminate infection. By contrast, the recognition and elimination of tumours by the immune system is much less effective. The main reasons for this difference are: i) the immune system is trained not to respond to our own tissues. Despite tumour cells undergoing uncontrolled growth, they are otherwise highly similar to normal cells and so recognised poorly by the immune system, (ii) the immune system can detect the presence of viruses, bacteria and other infections leading to full activation of the immune system. Tumour cells do not activate the immune system in this way. The weak immune responses to tumours are mediated by a type of white blood cell called T lymphocytes which can recognise the small differences between normal and tumour cells.

The objective of this project is two-fold. First, we will</td></tr>
</table>

	investigate the mechanisms of antigen recognition by T lymphocytes which are currently incompletely characterised. Second, using a novel approach we have recently developed, T lymphocyte recognition of human tumour cells will be optimised by altering the structure of the cell surface molecule used to detect tumour 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)?	T lymphocytes play a central role in the immune system through both killing infected cells and coordinating other cell populations involved in the immune response. This project will increase our basic understanding of how T cells recognise infected cells and some tumour cells which is more complex than originally thought and currently not fully understood. The transfer of T cells into patients (cell therapy) to target tumours is a promising approach but requires optimal recognition of the tumour cells. A key advantage of cell therapy over conventional approaches is its specificity for the tumour and avoidance of harmful side-effects. We will apply a novel approach we have recently published to identify variants of the receptors (used to recognise antigen) of tumour specific T lymphocytes. This approach has the potential to improve the recognition and elimination of tumours following cell therapy. We have an established track record in the area of T cell research and the work proposed is of direct relevance to human disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Only adult mice (wild type or genetically-modified) will be used for this project. We estimate that for all the procedures outlined in the application up to 1805 mice per year will be bred and 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	The majority of animals used will carry non-harmful genetic modifications and will be used for breeding. None of the experiments planned under this project involve procedures that are expected to cause the mice severe distress or discomfort. All procedures have been designed to detect any animals which

end?	appear to be suffering and if required humanely cull them. A small proportion of animals (<10%) will undergo mild/moderate surgical procedure and will suffer no other adverse effect than those associated with post-operative recovery. To minimise any associated suffering we have taken veterinary advice, will utilise anaesthesia and analgesia and terminate experiments early if required. Some animals (<6%) will be exposed to a tumour challenge For the mice undergoing this procedure, additional humane end points have been adopted to avoid unnecessary suffering. In the experiments proposed, the mice will develop either minor or, in the majority of cases, no symptoms before being humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In vitro experiments using tissues from experimental animals at the end of the procedures are highly informative and an essential element which will be used extensively throughout this project. The immune system is a highly complex involving multiple tissues, cells and molecules. Whilst invaluable, in vitro systems cannot replicate in vivo immune responses. Use of refined animal models is thus the only valid way of both advancing basic understanding of the immune system and for evaluation of novel approaches for immunomodulation of relevance to the treatment of human disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The main new factor in minimising the number of mice used in this project is our published development of a novel approach for producing very large numbers of variant T cell receptors (and other molecules) within individual mice. This approach will be applied to both objectives of the project to hugely increase the data which can be produced from each experimental animal. The following measures will be routinely adopted

	for minimising the number of animals used and deriving robust data: 1. Mouse colonies will be closely monitored to avoid excess animals. 2. Careful design of in vivo experiments will ensure the number of animals is kept to a minimum whilst providing valid conclusions. Appropriate statistical analyses will be applied. 3. In the majority of experiments, post mortem tissue will be subject to invaluable in vitro analyses.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use mouse models as this species has an immune system which is highly similar to human making it relevant for understanding the human immune system in health and disease. The availability of inbred, genetically modified mouse strains and reagents for analysing immune responses further strengthens its utility in research. As described above, our published development of a novel approach for producing very large numbers of variant T cell receptors within individual mice represents a substantial experimental refinement. This approach will be applied to both objectives of the project which could not be otherwise achieved. For measuring immune responses to transplants, we will exclusively use a non-surgical method in preference to surgical skin grafting. Appropriate guidelines for good practice will be followed. Animals will be inspected regularly to ensure general well-being and any animal showing signs of adverse effects will be humanely killed. The named veterinary surgeon and/or animal care and welfare officer will be consulted for advice where appropriate.

<table>
<tr><td>Project 18</td><td>Antibody production in rodents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Antibody Immunisation</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></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>The purpose of this service licence is to produce antibodies in the bloods and tissues of rodents, and supply these bloods and tissues to research establishments that lack the expertise, capacity or facilities in their own establishments to conduct the work.

Requirements for the antibodies are specific to each research program, and may support a broad range of objectives, specific to the client. For example: the characterisation, location and expression patterns of novel proteins identified during research, creation of targeted therapeutic compounds or development of novel diagnostic assays. Antibodies will only be produced if they do not currently exist, or those that do exist are of poor quality.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or</td><td>This provision of these services under this licence will allow important programs of scientific research to progress more rapidly, and thus increase the speed at which new potential therapies for human</td></tr>
</table>

animals could benefit from the project)?	and animal disease can be identified and developed. The provision of this type of centralised service is beneficial because it facilitates the development of a high level of expertise in the staff performing the service.
What species and approximate numbers of animals do you expect to use over what period of time?	The numbers of animals used is not expected to exceed 6,000 mice and 2,000 rats over 5 years and will either be bred from or supplied for studies.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The project authorises the immunisation of rodents to produce antibodies for future research. Most of the rodents are expected to experience no more than mild clinical signs. A small number may develop clinical signs due to adverse side effects of the antigens, which may be controlled by special care, veterinary treatment or by killing the animal if it appears to be developing adverse effects which are worse than predicted. Anaesthetics will be used as advised by a veterinary surgeon. At the end of the immunisation protocol the majority of rodents will be humanely killed by a schedule 1 method prior to tissue and blood collection. However, on rare occasions there may be situations where collection of samples in dead animal would not be compatible with the research objectives, or where this would increase the total number of mice used on a project. In these circumstances, where there are reduction benefits, collection of bloods and/or tissues under terminal anaesthesia may be used. For example, where aseptic collection of blood for polyclonal antibody production requires maximisation of total blood collection from each mouse, collection of blood under terminal anaesthesia at the end of the protocol will allow for a reduction in total number of mice used. A small number of mice may be shipped to a bone fide establishment for continued use for example where transit time for transportation of terminal samples could compromise the quality of the specimens.
Application of the 3Rs	
1. Replacement State why you need to use	No effective method for producing antibodies commercially without the use of animals is available. Cell culture techniques which allow the

animals and why you cannot use non-animal alternatives	production of antibody fragments do exist; however such techniques are still experimental and have an uncertain yield, efficacy, and antibody function. Tissue studies and computer simulations can assist in scientific research, although these procedures do not produce the bio-active antibodies that can be used to detect a wide array of proteins. Consequently, animal use is essential.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Working with customers and colleagues, we intend to continually assess the appropriateness of the techniques and equipment utilised for these protocols. Accurate, standard and concise collection of rodent information and program requirements with data will be sought from clients, suppliers and standard databases. Under this Service Licence, it is our responsibility to ensure that clients have given consideration to the use of non-sentient alternatives and identifying the most appropriate reduction strategies for research work. This will be achieved through the regular assessment of web resources (for example http://www.frame.org.uk and http://www.nc3rs.org.uk/), review of relevant journals, client meetings, attendance at regular industry meetings and symposia (for example LASA) and through the review of internal data. We will continue to review our facilities, training and processes in response to information on best practice in the use of animals in scientific 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.	Mice and rats will be used. Rodents are the lowest vertebrate group in which antibodies can be produced. Newer approaches, such as phage display libraries and transgenic mice, have proven much more successful in generating fully human antibodies, and there is every indication that continuing advances in these protocols will make the process more refined with improved outcomes. Working with customers and colleagues we intend to continually assess the appropriateness of the

	techniques and equipment utilised for these protocols. We will continue to review our facilities, training and processes in response to information on best practice for antibody production for continual refinement. Complete records of the health screening and welfare observations will be maintained. The quality will be assessed via retrospective review. Rodents will be observed appropriately to ensure they are maintained to humane endpoints as detailed in this licence. Our staff undergo their own continual professional development programmes and keep abreast of new developments which may facilitate refinement by attendance at meetings and conferences and by following the activities of organisations such as the NC3Rs.

Project 19	**Adjuvant effects of lipid-coated particles**	
Key Words (max. 5 words)	Adjuvant effects: lipid-coated	
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
	X	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 purpose of this licence is to compare the immunornodulatory effects of a previous model of synthetic lipid-carrier conjugate with the proposed, new model of natural vegetable lipids-Kaolin (natural) carrier by: 1. Assessment of synthetic (previous model) *vs.* natural (vegetable) (proposed model) lipid. 2. Assessment of the carrier of the lipid (silica-previous model *vs.* kaolin-proposed model) 3. Dose-response tests The study results will allow the applicant to assess the potential of testing the natural lipids-carrier conjugate further, i.e. on target (farm) animals, as a feed material aiming to improve the health and welfare of the said animals.	
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)?	• Comparison of immunomodulatory effects of previous model of synthetic lipid-carrier conjugate with proposed model of natural vegetable lipids-Kaolin (natural) carrier conjugate. • Provided that the beneficial effects of the initial model are also achieved with the natural conjugate (proposed) model, further studies on target (farm) animals will be carried out with the view of developing	

	a feed material that could: a. Improve immune responsiveness of animals. b. Improve well-being/welfare of farm animals leading to more rapid weight gain and/or productivity and more efficient animal production. c. Benefit animal farming, especially organic farms through use of a natural feed additive and possibly reducing the dependence on use of antibiotics. d. Reduce the dependence on antibiotics. This will lower production costs and help counter antibiotic resistance.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, up to 500 over a 3 year period. The number of mice accounts for using different strains of mice, in addition to Balb/c, to confirm that any effects seen are not strain- dependent.
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?	No adverse effects and mild severity expected (based on expected of previous, similar studies). All animals will be 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	This will be a comparative study to investigate efficacy of natural lipids-carrier conjugates *versus* that of synthetic lipids-carrier conjugates. What is sought is the assessment of the immune effect these conjugates have and as this is an effect on the whole body, animals, i.e. mice have to be used. In addition, initial studies using synthetic lipids-carrier conjugates were conducted in mice hence the data needs to be obtained using a reproducible animal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals employed correspond to previous studies and are minimal, but have proved adequate to detect statistically significant differences in immune response. More specifically the number of animals per group (6) corresponds directly with that employed in previous work titled “Synthetic lipids as an effective mucosal adjuvant”. In that work IgA antibodies were detected in saliva, vaginal washes and serum and the levels of salivary and vaginal antibodies detected in the KLH-lipid group compared

	with the KLH alone were significantly enhanced ($p < 0.01$). Serum IgG, IgA and 1gM antibodies were also raised and comparable with CT immunised animals ($p < 0.01$). Furthermore on-going statistical advice and assistance will be provided by a third party consultant 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, being the least sentient animals, will be used in this study to test the concept prior to trials in larger species (e.g. farm animals). Species, strain (Mice, BaIb/C), models and methods are chosen as described above, based on the species, models and methods used in past studies. The study will use minimal, but sufficient, numbers of animals, and will be minimally invasive (oral dosing of test substances). Administering the test substances will not be stressful and no adverse effects are anticipated.

<table>
<tr><td>Project 20</td><td>Antibodies research, diagnostics and therapeutics use</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Monoclonal, polyclonal, antibodies</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></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></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></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>

To produce antibodies for use in the immunodiagnosis of human and animal diseases, metabolic disorders, detection of drugs of abuse, cancer screening, and environmental testing.
To produce monoclonal antibodies for use as biotherapeutic agents for treatment of a wide range of diseases and physiological or biochemical disorders such as cancer.
During the tenure of this licence we would expect to produce 3-5 monoclonal and 5-10 polyclonal antibodies.

</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 project is expected to create new clinical and diagnostic antibody probes to advance knowledge or treatment of human and animal diseases, metabolic disorders, detection of drugs of abuse, cancer screening, and environmental testing for microbial or chemical contaminants and pollutants. Previous projects have resulted in antibodies that are used in stem cell research, and several</td></tr>
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	diagnostic tests.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, up to 100 over a 5 year period Guinea Pigs, up to 20 over a 5 year period Rabbits, up to 20 over a 5 year period Sheep, up to 10 over a 5 year period Goats, up to 10 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?	No adverse effects and moderate severity expected (based on experience of previous, similar studies). Animals will be monitored daily for local inflammatory reactions caused by adjuvants. In particular, if there is any evidence of abnormal behaviour and/or ulceration that breaks the skin; affected animals will be killed. Similarly, when administering Freund's Incomplete Adjuvant via the intra-peritoneal route, animals will be monitored for evidence of peritonitis and killed immediately if peritonitis occurs. Animals will be killed by: A schedule 1 method; exsanguination; or removal of organs and tissues. Animals may be rehomed if suitable,subject to conditions set out in section 17A of The Animals(Scientific Procedures)Act 1986(as amended)
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose is to produce whole antibody proteins. This requires an immune response which can only be generated in, or from, live animals. There is no alternative non-animal model available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers of animals employed are based on previous work, which gave good antibody responses with a small number of animals.
3. Refinement Explain the choice of species and why the animal model(s)	Previous studies with all candidate species have given rise to strong and reproducible antibody responses. Mice, rabbits and sheep are often 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.	species of choice for producing monoclonal (mice) and polyclonal antibodies. *Termination* Humane end points for an immunisation programme will be either when two consecutive blood samples show the serum antibody level has reached a plateau, or when the inoculation regime reaches the maximum number of challenges, namely x5 sequential inoculations.

<table>
<tr><td>Project 21</td><td>Understanding perturbation of innate immunity in vascular inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Vascular disease, inflammation, innate immunity</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>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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We would like to understand how common features of a ‘Western’ diet (eg. high fat) impact on the immune system in vascular disease. We will also explore how these common mechanisms can cause increased risk of infection and worsen infection outcome.</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>Atherosclerosis and related vascular diseases including chronic kidney disease and autoimmune disease account for the majority of deaths in the UK. In all cases, a high fat diet and elevated blood cholesterol is known to worsen these conditions. This research will help unravel the impact of diet on how the immune system contributes and responds to these diseases and potentially lead to discovery of novel biological therapies.</td></tr>
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What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6000 mice and 3000 rats 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 maximum severity for all animals is moderate. Any adverse effects from procedures will be carefully monitored. The majority of procedures involve the dietary modification of genetic knockout animals to study cardiovascular disease. Some surgical protocols are used to study models of kidney disease. All animals will be humanely culled at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation and vascular disease are complex dynamic tissue processes that cannot be reproduced by growing cells in a lab. Nevertheless, aspects of cellular function and intercellular interactions can be studied in such models, with the reduced experimental conditions often providing more clear-cut conclusions than are possible in an animal model. Our approach is to therefore to use animal and cell culture experiments in a complementary manner and to replace animal work with cell culture methodology wherever possible, for example using cultured cells exposed to different forms of blood flow in a specialist chamber, to mimic the conditions found in a vein or artery.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The reduction of animals will always be used where possible. Using cutting-edge technology, we are able to track labelled cells in animal models of disease using whole body imaging without any requirement for surgery. This means that the same mice can be anaesthetised and imaged many times with minimal stress, greatly decreasing the number of animals required.
3. Refinement	Mice and rats are used to accurately model human physiology and biology. All animals will

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.	receive pain relief to treat any apparent discomfort, and these drugs will always be given prior to any surgery. In this way we are continuously striving to make small changes in procedures to improve welfare.

Project 22	**Macrophage control of injury and repair**	
Key Words (max. 5 words)	Macrophage, kidney, inflammation, treatment	
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)	Acute kidney injury (AKI), chronic kidney disease (CKD) and hypertension are major contributors to human disease burden. We currently have no effective therapy for AKI. Although we can slow progression of some forms of CKD, it remains the major cause of dialysis requiring renal failure. Hypertension remains the major cause of stroke, cardiovascular disease and CKD. All of these diseases are associated with the presence of macrophages, a specialised white blood cell, however their role both injurious and beneficial has not been clearly established. This project aims to determine their role in renal injury and hypertension, determine whether macrophages can be used as cell therapy and develop new treatments that utilise the reparative potential of macrophages. It will also look at the role of stem cells in renal regeneration both to determine the mechanisms of repair and their potential as therapeutic targets.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or	*1.* Greater understanding of the natural history of renal disease and the mechanisms by which macrophages and stem cells affect this. 2. Establish the role of macrophages in the control of	

animals could benefit from the project)?	hypertension 3. Develop macrophage cell therapy for treatment of diseases such as acute kidney injury and chronic kidney disease. 4. Develop new drugs for clinical translational studies in renal disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 years of this project: Mice 1950 Rats 500
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 models chosen mirror human disease with the least disturbance to animal welfare. The majority of models will involve induction of mild disease, which will have no apparent effect on animal well-being. The models are chosen because they accurately mirror human diseases and insights obtained from them are directly related to human disease. ***Nephrotoxic nephritis (NTN)*** is a model of acute renal inflammation. It has been refined over many years to give reproducible levels of renal injury with the minimal side effects. As with human disease it is monitored by measuring the leakage of protein in the urine and serum creatinine. ***Adriamycin/puromycin nephropathy*** causes chronic kidney injury that mirrors the human diseases that cause persistent protein leakage in the urine. The level of inflammation is less marked than seen in NTN, and results in minimal side effects. ***Ischaemia rep erfusion injury (IRI)*** is the most common cause of acute kidney injury in humans. In this model the renal vasculature is clamped for a brief period and then blood flow restored. Animals recover rapidly from the procedure. Hypertension is one of the most important causes of cardiovascular disease. ***Hypertension*** is induced by modifying the diet (high salt normally) or infusing vasoconstrictors. This is tolerated without adverse effect. To monitor blood pressure telemetry devices can be inserted which enables measurement without handling. Some protocols will involve surgical procedures. These have potential to result in localised discomfort which will be prevented by appropriate use of anaesthetic and prophylactic analgesia. These

	experiments will be of moderate severity. 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	Kidney disease and hypertension are complex diseases which evolve progressively over an extended time and involve interaction between many cell types. This can currently only be studied in animal models. These models enable us to determine the role of macrophages and stem cells and develop new therapies. In vitro we can assess the effects of inflammatory mediators and hypoxia on individual cell types involved in kidney disease and assess the therapeutic benefits of drugs and modified cells. These experiments provide valuable mechanistic insights and confirm potential therapeutic approaches. These can then be assesses in the more complex in vivo environment. Also targets identified by in vivo experiments and assessment of human disease can then be taken back into cell culture models to further understand their role in disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies are designed to gain as much information as possible without compromising animal welfare. The models outlined above have robust readouts (e.g. serum creatinine, proteinuria, blood pressure) that are identical to that assessed in human disease. By obtaining sequential data from a single experiment we can optimise the information gained. Where possible we can use a cross-over design of experiment which enables each animal to act as its own control, which significantly reduces the number of animals required. We will also use modern imaging techniques such as renal ultrasound and/or IVIS imaging on lightly anaesthetised animals to provide non-invasive longitudinal information on development of injury. All of the experiments generate a range of readouts including biochemical, blood pressure, histology, immunohistochemistry and functional analyses from individual isolated cells. By maximising the data obtained only small numbers of animals will need to be used. We are normally looking for changes in significant clinical parameters such as serum creatinine, proteinuria, blood pressure or kidney cell death. This can then be used to perform a statistical

	power calculation aiming to determine the size of the experimental groups. From our previous experience this requires groups of ~6-8 for each condition.
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 models chosen are the closest to human disease with the minimum compromise to animal welfare. All the models are well characterised in rodents (mice and rats) with decades of international experience which have furthered our understanding and treatment of human disease. The use of these models allows the study of specific transgenic and knockout strains that optimise mechanistic insight. There is also extensive experience in correlating data from these models with relevant human diseases. All the models described above are well established. Suffering is kept to a minimum by careful experimental design, good surgical technique and supervision and use of aseptic techniques, anaesthesia and pen-operative analgesia. Where operations are required (e.g. IRI) pen-operative fluid is administered according to well-defined protocols. Post-operatively animals recover in temperature controlled individual cages with easy access to moistened food.

Project 23	**Role of Fat Associated Lymphoid Clusters in Metabolism and Immunity**	
Key Words (max. 5 words)	Immunity, Inflammation, Metabolism, Obesity	
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 immune system, which is primarily involved in the control of infections, is also very important for the regulation o metabolism and the good function of fatty tissues. However, he connection between metabolism and immune system is till poorly understood. Chronic inflammation in fatty tissue is important in obesity, but use of anti-inflammatory therapies has had very modest or no efficacy in restoring metabolic health. In this project, we want to understand what is the role played by the immune system in obesity and we want to design new strategies using the immune system to treat the metabolic dysfunction associated with obesity. We recently showed that immune clusters present in fatty tissue are involved in inflammation and immune responses against infection. In this project we want to expand our understanding of the immune and inflammatory roles of these clusters during infection and we want to define the role played by these immune clusters in metabolism and obesity. There is a scientific and clinical need to understand the link between immune system and metabolism and to develop new cures for obesity.	

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)?	Most of the post-industrialised and newly industrialised countries are facing an obesity epidemic and its associated pathologic conditions (type 2 diabetes and liver disease for example). This major health issue is threatening humane global health and there is today a serious need for effective therapies. This research will contribute to our understanding and knowledge of how the immune system control metabolism and fat tissue function. By defining the mechanisms underlying inflammation and the defence of the peritoneal cavity against infections this work will enhance our knowledge of immune responses and open new roads for the development of therapeutics tools in the treatment of inflammation. This work will pave the way to future translational studies looking at immune clusters in fatty tissue in humans and the therapeutic benefit of their targeting in inflammation and in obesity.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6,000 mice will be required to maintain the different colonies of genetically modified mice for the studies and generate sufficient experimental
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 be used in breeding programmes propose to do to the animals. Diets will be used to induce obesity and will not visibly affect the health or state of the animal. The inflammatory and infectious models to be used in this project are well tolerated; none cause mortality. Substances will be administered at doses known to be non toxic, based on experience and dosages reported in the literature, and at volumes in accordance with best practice. For blood sampling, to avoid hypovolaemia or anaemia, no. more than 10% of the total blood volume will be withdrawn on any one occasion and no more than 15% of the total blood volume in any 28-day period. Surgery will be performed to assess the function and formation of lymphoid clusters by transfer under the kidney capsule or by removal of the spleen or the omentum. Deaths resulting from anaesthesia or surgical complications are uncommon and will be minimised by correct dosing of anaesthetics, by accurate weighing and by maintenance o

	body temperature during and post surgery eg. use of heat pads. Pain will be controlled during surgery by general inaesthesia and post surgery by analgesics. Best practice guidelines for surgery/post-surgical care, anaesthesia and analgesia will be followed at all times. To understand the role of certain subsets of immune cells in inflammation and obesity we will perform immune cell depletion. This may increase the risk of infection and animals will be housed in barrier environments and may be treated prophylactically with antibiotics, under direction of a veterinary surgeon. To understand the function of molecules in inflammation and obesity we will perform bone marrow chimeras. Following irradiation animals will be at an increased risk for infection. This will be minimized by use of aseptic handling technique and housing in a barrier environment. In some cases the addition of antibiotics via the drinking water will be used to control potential infection, If animals exhibit clinical signs of infection, humane end points will be applied. Insulin tolerance tests (ITT). Hypoglycaemic shock (fitting, seizure) is a possible adverse effect. Animals will be monitored throughout the ITT and humanely killed if hypoglycaemic shock occurs. All procedures used are classified as moderate. However, in many instances the actual severity experienced by the animals will be mild, particularly in the breeding protocol. In he case of unexpected adverse effect, the animal will be humanely euthanised. At the end of the procedure, the animals will be humanely euthanized and tissue collected for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mice have been used as a model to understand immunology or more than 30 years and are an excellent model for the human immune system. In this project we want to understand he interaction between the immune system and metabolism, which cannot be modelled in a test tube and can only be accomplished *in vivo.* Given the wide range of genetically modified mice and the wealth of reagent available, these animals provide the best means for

	analysis. Nevertheless, maximal attention will be given to obtain information from *in vitro* and *in sillico* studies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use inbred mice, which reduce inter-animal variability and thus overall numbers required. We have carefully calculated the optimal sample size for the experiments described in this project to ensure statistical significance but use the minimum number of mice. We will consult a statistician when necessary. Where possible, a multi-factorial design will be used to increase power and reduce the overall number of animals required. *In vivo* imaging allows sequential non-invasive measurements within a single animal, increasing statistical power and reducing the number of animals required for experiments. The group and collaborators will discuss planned and current experiments in regular meetings to ensure the best utilisation of the tissue/data from 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.	In the proposed project, knockout mouse strains deficient in molecules involved in the function of specific immune cell subsets will be used. Among experimental species, mouse immune system is the best characterised with well-established markers for defined immune cell subsets and activation stages. This project also involves a model of obesity widely used to study obesity. Therefore, mice constitute the choice to answer the questions. Throughout, we will ensure that the least invasive methods of dosing and sampling are applied, including the use of anaesthesia for humane restraint when appropriate. For all surgical procedures, the less invasive and painful approach will be used, the appropriate anaesthesia will be chosen, and he best aseptic techniques will be used. All animals recovering from surgery will be carefully monitored and analgesia will be systematically given. All immune-compromised mice (GA strains lacking specific immune cell populations or mice that have been irradiated) will be housed in barrier environment to reduce the risk **of i**nfections. Humane end points will be determined by clinical signs including weight loss, inactivity, hunched posture, starey coat, dehydration etc. Where the severity of the combination

	of signs is in doubt advice of the named persons will be sought and taken. We will keep all surgical procedures under review and developed and use more refined ones where possible.

<table>
<tr><td>Project 24</td><td colspan="3">Immunological defence against bacterial pathogens</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Tuberculosis, melioidosis, immunity, vaccines</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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></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">Infections with bacteria are a current and increasing threat to human health. The purpose of this application is to study two infections in particular- tuberculosis (caused by Mycobacterium tuberculosis) and melioidosis (caused by Burkholderia pseudomallei). Tuberculosis is one of the leading causes of death by infection worldwide, combining with HIV to kill many millions of people each year. In contrast, melioidosis is not a global health problem but is a major cause of severe, lethal infection in SE Asia and Northern Australia, with increasing reports of infections in other tropical countries. In both cases, infection kills if untreated, antibiotics are available but treatment takes 4-6 months to be effective (and often fails) and there is no available vaccine to prevent the majority of human cases. Furthermore, both infections target the lung as a site of exposure and tissue damage</td></tr>
</table>

	and both have the ability to live within cells of our immune system. The objectives of this application are to use models of infection with these bacteria in mice, which are known to mimic many of the clinical features of the human disease, to understand more about how our immune systems respond to these organisms in order to build better vaccines and treatments.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits that will be generated from this study are: • Identification of genes which determine the virulence of *M. tuberculosis* and *B. pseudomallei in vivo*. These are a necessary prerequisite for the generation of rationally attenuated live vaccines as well as providing targets for immune intervention. • A better understanding of how the immune response controls these infections. The information obtained here will be directly applicable to other intracellular organisms such as *Salmonella*, *Toxoplasma*, *Leishmania* and others. • Development of generic immunotherapeutic approaches which can be used in conjunction with antibiotics to reduce mortality. • Generation of novel vaccines against tuberculosis and melioidosis. • Further development of non invasive imaging to allowing us to reduce the numbers of animals needed in these experiments in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 12,250 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	The animal models to be used are designed to mimic the infections that *M. tuberculosis*, *Burkholderia pseudomallei* (and *Listeria monocytogenese*) cause in humans. For the majority of experiments, using genetically modified bacteria with deletions in key virulence

end?	genes, or infection with wild type bacteria into mice given novel vaccines is likely to cause either no obvious clinical signs or infection of moderate severity, with signs such as minor but not extensive weight loss, transient piloerection or huddling. However, in the minority of experiments, control animals in which wild type bacteria or untreated mice are used, the infection will proceed as it does in humans to extensive weight loss and severe infection with the potential for death. In these cases, mice will be culled when reaching a defined humane endpoint which allows us to assess the true protective efficacy of our interventions with the least amount of distress to the animals. At the termination of each 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	The purpose of this research plan is to study the interaction of pathogens with the host immune system in order to identify fundamental mechanisms in host resistance and to direct studies on obtaining new prophylactic and therapeutic regimens. Such studies cannot currently be mimicked in cell culture models and require vaccination/treatment of live animals, followed by challenge with the Mycobacteria or Burkholderia spp of interest and assay of host immune responses and health.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments are set up to minimise bias by randomly allocating animals to each treatment group, ensuring that housing and all subsequent treatments are done in random order and that where possible researchers are blinded with respect to treatment groups. We have access to statisticians to discuss new or refined experimental designs. The number of mice used for these experiments will be the minimum number needed to provide sufficient cells/tissues for assays of immune function and to achieve adequate statistical power. The number of animals will be significantly reduced by our development and

	usage of non invasive imaging technologies. In all cases, sample sizes will be continuously monitored and adjusted in the light of analysis of the data as it becomes available. In each experiment, it will always be necessary to have a contemporaneous control group. However, analysis of multiple gene knockouts, vaccination strategies, drug doses or other variables will be routinely performed during the same experiment so that control animals can be shared, thereby reducing the number of animals required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The models chosen have been selected to cause least overall harm whilst still achieving the objectives. Mice are widely accepted as models for human infectious diseases, and this is particularly true for both MTB and Bps infections. Other larger animals such as hamsters, guinea pigs, rabbits etc can be used to model infection with these bacteria but the immune systems in these species are less amenable to study due to the lack of defined genetic knockout strains and immune reagents. The key bacteria strains we will use are derived from human cases of disease and the infection routes we will use mimic the natural route of exposure of humans living in endemic areas. Our goal is to mimic the human disease as closely as possible in order to better understand the nature of immunity and also to provide the most realistic setting for evaluation of new drugs and vaccines in the murine models. Use of non invasive imaging throughout the project will reduce the total number of animals used and in some cases determine an endpoint for experiments before the onset of visible signs and symptoms.

<table>
<tr><td>Project 25</td><td>Host and bacterial factors interactions in tuberculosis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>M. tuberculosis, mouse,</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>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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To ascertain the importance of host and or pathogen genes in M. tuberculosis infection.</td></tr>
<tr><td rowspan="2">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>Each year, 9-10 million people develop tuberculosis for the first time. Approximately 2 million individuals die from TB every year, 0.2 million of which occur in HIV-coinfected patients, and TB continues to be the leading cause of death in HIV-infected individuals in Africa. Further aggravating the current situation is the rising frequency of multi-drug resistant TB (MDR-TB) strains, which are resistant to the front-line drugs isoniazid and rifampicin. Clearly this is an urgent need for new vaccines and anti-tuberculosis antibiotics, which can be made achievable by the knowledge obtained from using animal models of tuberculosis.</td></tr>
<tr><td>Tuberculosis is a very complex disease where the contribution of both genetic factors in the host and</td></tr>
</table>

	virulence factors in the bacteria determines the outcome of infection (e.g. active vs. latent infection). Utilisation of the mouse TB model for infection will aid in the assessment of these factors. These studies will therefore address current gaps and will advance our knowledge in prevention and/or treatment and key research areas such as novel and combinatorial strategies for prevention, novel therapeutic and/or curative approaches, development of models for disease progression and host-pathogen interaction in human tuberculosis.
What species and approximate numbers of animals do you expect to use over what period of time?	*Mus musculus* 4110 mice per year
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Based on our experience, we anticipate that the symptoms associated with these procedures will be mild (95%) to moderate (5%). It is not always possible to predict outcomes when new infection strains or combinations are utilised. In those circumstances extra observations will be made. Those instances where animals may develop moderate severity signs as part of a procedure are described below. Animals exhibiting any unexpected harmful abnormal phenotypes will be killed and advice will be promptly sought from the NVS and local Home Office Inspector.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Initially, the role of specific genes and compounds, which can affect the induction of an immune response and/or growth of a pathogen, will be determined in *in vitro* and *ex vivo* studies. Ultimately, however, the immunological and immunopathological investigations, and particularly vaccine and antimicrobial effectiveness cannot be carried out without the use of animals, since the host's immune system cannot be entirely mimicked by any *in vitro* assay. Furthermore, although all compounds will be selected for *in vivo* testing based on evidence of activity in relevant *in vitro* assays, this cannot replace the *in vivo* tests under the physiological conditions of an infection, as potent *in vitro* activity might not translate into an *in vivo* activity. Thus the information obtained from *in vitro* studies will then be used for *in vivo* studies. To achieve the objectives of this project, we

	propose to use the laboratory mouse as the model organism. The mouse model is the best-characterised model for these studies, with many features applicable to human infection. Their immune responses are well defined and the technology enabling sophisticated manipulations of the haematopoietic and immune system is highly developed. Mouse transgenic and knockout techniques are well established; mice have a relatively short generation time; its haematopoietic system has been extensively studied and, in addition to the accumulated knowledge, there exists a vast array of reagents that facilitate the studies to a level unknown for many other organisms. To our knowledge, no other species of lesser sentience can fulfil the requirements of this project to the same extent as the mouse.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For most of the quantitative experiments, design will be based in ARRIVE guidelines and sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80% and at least practicable difference between groups of 20%. Otherwise we will use the minimum number of animals to provide an adequate description, generally on the basis of previous experience (our own or from the literature). Pilot experiments will use between 5-8 mice per group, which should be sufficient if a significant result is obtained.
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.	Virulence or pathogenicity of *M. tuberculosis* will be assessed most frequently as morbidity, which is quantifying changes in physiological parameters and clinical symptoms relevant to the infection. Every effort will be made to prevent infection-associated deaths. In most of our experiments, morbidity will be the pathogenicity measure and we will go to great lengths to minimise the possibility of death. This will be achieved by close monitoring of clinical symptoms and physiological parameters during the course of the infection. When clinical symptoms reach a moderate level or the physiological parameters exceed the values detailed in protocols, mice will be killed by a Schedule 1 method.

<table>
<tr><td>Project 26</td><td colspan="3">Understanding the role of signalling molecules in immune cells</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Immunology, signalling, gene expression, autoimmunity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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></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>
</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">While significant advances have been made in our ability to understand functioning of the immune system, our knowledge of some aspects of immunity, especially at the level of what occurs within an individual cell, is still very basic. Given the critical roles immune cells play in fighting infection and, when not correctly regulated, in the development of many common diseases, this is an important gap in our knowledge. To address this we will use genetically modified mice to understand some important unanswered questions about the function of specific proteins in the immune system.</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 colspan="3">Immune-mediated diseases (autoimmune disorders, leukaemia, lymphoma, immunodeficiency diseases) represent a major healthcare issue and despite advances are difficult to treat effectively without development of adverse side-effects or drug</td></tr>
</table>

project)?	resistance. In addition a significant proportion of patients do not respond to current drugs, and for some that do respond the drugs become less effective over time. There is therefore a pressing need to develop better drugs for these conditions. These diseases are driven by derangement of the normal control mechanisms that regulate the development and function of specific immune cells. In order to understand how these diseases arise, we need to better understand how the immune system operates normally and how it goes wrong during the development of disease. This project aims to use genetically modified mice to understand how specific molecules control the development and function of immune cells. In this way, we hope to identify new targets that can be used to develop novel drugs to treat immune-mediated diseases and/or boost vaccine strategies.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will only use mice. Most of the mice will be used either for the breeding of gene targeted mouse lines or the provision of mice for the isolation of cells or tissue for further study. Up to 6000 mice will be used for this over 5 years of the project. A subset of these, less than 10%, will be used in experimental protocols to examine their immune function.
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 genetically altered mice used under this licence will not exhibit adverse welfare effects. A small number of the minority that undergo an experimental intervention (max 10%) may reach moderate severity limits; none should reach substantial severity limits. Immunisation protocols, to test the ability of the immune system to respond to defined agents may induce flu-like signs, but these are expected to resolve fully. Skin sensitivity assays may induce an eczema-like condition, but this will be limited to the very small area of skin on which the sensitising agents is painted. In some cases, in order to determine whether a genetic alteration exerts its effects directly within the cells of the immune system or within other cells with which that system interacts, it will be necessary to “knock-down” the immune system with irradiation, and then to “reconstitute” it with cells (normal or genetically

	altered) derived from another animal. There can betransient weight loss / increased risk of microbial infection post-irradiation, but good husbandry will ensure that these resolve promptly and without lasting harm. Finally, we wish to study animal models of arthritis, a disease primarily of the immune system. Animals in these studies may exhibit sluggishness, ruffled fur and joint swelling. We will abide by a careful scoring of the affected limbs to limit the severity of these experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Work using cultured cells cannot mimic the complex physiology of the mammalian immune system. Furthermore, experiments using cell lines often do not adequately reflect physiological responses and can sometimes lead to erroneous conclusions. Therefore it is necessary to do some experiments that involve the use of laboratory animals. Mice will be used, as this allows the use of genetically targeted mice to study the function of specific genes in immune cells. The mouse immune system bears extensive similarities to that of humans and these similarities far outweigh any differences. There also exists a tremendous arsenal of laboratory reagents which allow us to detect and manipulate mouse blood cells. Therefore, we can extract more data from each experiment using mice than we can with any other model organism. For the majority of this work we will study cells isolated from the immune system of these mice, as this will allow us to complete much of our work without the need for experiments in live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Breeding programs will be kept to the minimum required to maintain the line and provide mice for experiments and cell isolation. Cryopreservation will be used to archive lines that are not required for on-going research. While whenever possible we will use studies on isolated cells or tissue, due to the complex nature of the immune system it will be necessary to test some of the predictions made from these studies in

	mice. For experimental models accepted statistical methods will be used to establish the minimum group sizes necessary for the work. In this way we will minimise the numbers of animals in which a direct experimental intervention is required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice will be used due to the ability to carry out gene targeting and the availability of research reagents. The majority of the mouse lines used for this project do not have apparent adverse effects on the animal's welfare. If this this does occur, protocols will be put in place in conjunction with the named vet in order to minimise any adverse effects. For *in vivo* experiments, end points with the lowest severity possible to answer the scientific questions will be selected.

Project 27	**Identification and characterisation of haematpoietic stem cells**	
Key Words (max. 5 words)	Blood, transgenic, stem-cells, therapy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 blood stem cell resides in the bone marrow of the adult and supplies all lineages of the blood system throughout life. We aim to gain an understanding of the signalling processes in the embryo that lead to the production of the first and subsequent blood stem cells. We have developed novel culture systems that allow us to study, for the first time, the development of blood stem cells in vitro from their precursors. We now aim to use a variety of approaches to investigate which genes are significant for blood stem cell development, including those in the cells of the environmental niches as well as blood stem cells themselves. We are also advancing protocols for development of blood stem cells from embryonic stem cells in defined culture systems.	
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 knowledge will help in generating culture based systems for producing blood stem cells or more differentiated blood cells, from human embryonic stem cells (or reprogrammed “stem cells”) which will have therapeutic uses in treating haematological disorders or leukaemia.	

What species and approximate numbers of animals do you expect to use over what period of time?	65,000 mice 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?	We expect few adverse effects in our breeding colony, including transgenic mice, which is the source of most of the experimç4htal material. . The use of irradiated recipients to detect functional blood stem cells is unavoidable since this is the only method available. This protocol has a moderate severity and the recipient animals are carefully monitored and are culled at the end-point, which is typically after 16 weeks, or sooner if they display signs of sickness where recovery is not expected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	**The development of blood stem cells** in mouse models is well characterized and coupled with the power of transgenic technologies, there is no better system to investigate genes important in determining blood stem cells in mammals. The long-term repopulation assay is also the only means of detecting the presence of functional blood stem cells and no alternatives are available.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals are bred to achieve a colony size that is sufficient to meet our research need. We have considerable experience in blood stem cell transplantation. Coupled with statistical methods we ensure that an appropriate number of animals are used gain a meaningful data sets. If our aims of extending knowledge we gain from the embryo studies can be used to inform design of culture based differentiation protocols of embryonic stem cells, we will ultimately have methods to drive the production of blood stem cells in culture and reduce the requirement for 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	Mice are the most accessible mammals for studies on stem cells due to the ease and isolation of stem cells. We have a state-of-the-art animal facility and all researchers are fully trained in the procedures they perform in their experiments and will follow recommendations given by the animal facility staff to further refine techniques.

measures you will take to minimise welfare costs (harms) to the animals.	In the transplantation experiments, the irradiation dose is given in two parts in order to ablate endogenous HSCs but to minimize stress. Mice are maintained on antibiotics after irradiation to prevent infection and closely monitored.

<table>
<tr><td>Project 28</td><td>Role of Pattern Recognition Receptors In Immunity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunity; Infection; microbes; autoimmunity</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></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>Our objective is to understand how cell based molecules, called pathogen recognition receptors (PRRs), enable the functioning of our immune system during infection with medically relevant pathogens and during the development of autoimmune diseases of the joints (arthritis), lung (asthma), eye and kidneys). We also need to develop tools such as antibodies and transgenic T-cells to study PRRs in these systems. These tools are made widely available to the scientific community following appropriate material transfer agreements, to achieve maximum impact.

Experimentally, in order to determine the role of a particular PRR, we compare immune responses of normal mice to those of mice genetically deficient in that PRR. In this way we can determine which PRRs are important in the control of particular infections or in the prevention of certain autoimmune diseases. This strategy will help us to understand the disease process and the way in which the body attempts to control it.</td></tr>
</table>

	Understanding these mechanisms is the foundation for being able to identify avenues for new and better treatments in humans.
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 benefit of our research is the furthering of scientific knowledge of the underlying molecular and cellular mechanisms of immunity. This advancement of knowledge will identify novel avenues for future therapies to improve public health in the longer term. Our work has already led to substantial academic advances, as evidenced by publication in high impact journals, and has also directly led to an understanding of disease susceptibility and to novel therapies in man.
What species and approximate numbers of animals do you expect to use over what period of time?	20,000 mice and 100 rats during the 5 year PPL. These numbers represent the theoretical maximum, and in practice will likely be less. Estimates are based on the required group sizes for experiments, experience on how many experiments are required to complete the studies of the number of researchers operating under this PPL.
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 are injected with natural or synthetic substances which cause immune responses that can induce diseases which we need to study, including inflammation, eye disease, arthritis, asthma and renal disease. We also retain a small number of mice for aging studies. While the majority (approximately 80%) of mice undergoing these procedures will have no significant adverse effects, in some studies the mice can become moderately unwell for a few days. These animals may show weight loss, become less active, have ruffled fur and appear hunched. During models of arthritis, their limb joints may temporarily become slightly red and swollen, and the mice may limp when walking on solid surfaces. For models of infectious disease, mice are infected with medically relevant infectious microbes. In most studies the mice can become very unwell and therefore this protocol is listed as *severe*. Animals will show up to 30% weight loss, become less active and isolated, have ruffled fur and appear hunched. In all our experiments, mice which become ill/lame

	are humanely culled as soon as the scientific outcomes have been achieved within defined clinical endpoints, and are closely monitored during the course of the experiment. We have already established such a monitoring system for our experimental models in consultation with the senior animal technicians and the veterinary surgeon. All animals are killed at the end of the study and tissues analysed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our mouse models (*in vivo*) are for studying clinical diseases for which there are no other laboratory (*in vitro)* alternatives. Experiments with cultured cells in dishes cannot recreate the complex cellular interactions required to fight disease *in vivo*. Wherever possible we use cell lines, human cells, or other *in vitro* methodologies (as they are developed), particularly for understanding the molecular mechanisms of immune cells. Approximately 20 % of our work is carried out *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction in animal use will be achieved by: -multiple readouts from animals *post mortem*. -use of appropriate group sizes to obtain statistical significant results. -use of inbred strains of age/gender matched mice which reduces intra-group variation. -optimisation of procedures and protocols to minimise experimental variability. -freezing mouse embryos/sperm for strains not required for some time.
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	Since mice are the worldwide standard laboratory animal model for immunological study. Most reagents available for this species, as are genetically altered animals. We use rats to generate rat anti-mouse monoclonal antibody reagents. The use of non-mammalian species will not provide the crucial insights required to understand human resistance to disease.

(harms) to the animals.	We optimise and refine our techniques to ensure maximum output from the minimal number of animals, in part through literature searches and discussions with other investigators to ensure the latest practices are employed. We make use of small pilot experiments in new areas to gain insight into potential adverse effects, and define humane endpoints. The experimental data that we generate feeds back into planning for future refinement. Where there is potential suffering for the animals, this is minimised by ensuring that all personal licence holders are appropriately skilled and trained in our clinical monitoring systems to identify humane end points, and through the use of techniques for the alleviation of pain and distress (e.g. analgesia). We are continuously refining our clinical monitoring systems. This is being achieved through observations made by the experimenters and animal care staff and discussions with the senior animal care technicians and the veterinary surgeons. Our studies make use of killed pathogens, where possible, to minimise the effects on the wellbeing of the animals.

<table>
<tr><td>Project 29</td><td colspan="3">Production of potent monoclonal antibodies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Hybridoma, monoclonal, antibody, therapeutic, reagent</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></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 colspan="3">This project licence will be used to generate effective medicines and reagents to support clinical programs and will also help improve the method currently used to produce these medicines and reagents.</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">This project will generate high quality medicines which will be used to treat patients who have diseases such as cancer, asthma, metabolic disease, pain and neurodegenerative diseases, and a number of other diseases across different therapy areas.</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">Over 5 years we would expect to use 1500 mice 300 rats</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected</td><td colspan="3">The protocol used is a standard procedure for the production of monoclonal antibodies .The protocol has been categorised as mild because the procedures undertaken are not expected to result in</td></tr>
</table>

level of severity? What will happen to the animals at the end?	the animals developing any clinical signs of disease or ill health. At the end all animals will be killed by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The approach used in this project is, in some cases, the only way to generate effective antibody reagents and antibody medicines for different types of disease. For example, it is challenging to generate medicines to disease targets which are found on the surface of cells using other technologies. A review of the most appropriate approach for generating our medicines will be carried out at the beginning of each programme.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use our experiences gained from the previous licence to guide the design of our studies. We will always plan to use the minimal number of animals for each experiment and will constantly analyse the data that we generate to see if further animal reductions can be made for the future.
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 and rats are small and easily handled species with a highly characterised immune system and well defined biology. Mice are also short lived, have rapid generation times and are easier to look after than other larger animals • The nature of the antibody response in mice is very well characterised. The route of injections used in this project have all been shown to cause no adverse effects whilst inducing effective antibody responses in most cases • Rats will tend to be used when antibodies specific for a mouse target are required. • The route of injection used in this project have all been shown to cause no adverse effects whilst inducing effective antibody responses in most cases

Project 30	**Therapies for Infectious Disease and Cancer**	
Key Words (max. 5 words)	Cancer, influenza, tuberculosis, vaccine	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	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)	Infectious disease and cancer remain major health burdens worldwide. We are performing research for the development of vaccines that can be used to prevent or treat infectious disease or 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)?	Once we have vaccines that work in animal experiments we will publish our data. Then they can be tested in clinical trials with the ultimate goal of developing new therapies. Our research will also advance basic knowledge of the immune system in health and disease.	
What species and approximate numbers of animals do you expect to use over what period of time?	About 250 mice per year for 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 animals will experience some of the symptoms of the diseases that we are trying to treat. For example a small number of the mice will experience moderate flu symptoms such as weight loss. These mice will be humanely killed before the symptoms progress further. BCG does not cause any ill effects in mice. A number of mice will also experience moderate discomfort from tumours but will be humanely killed before discomfort progresses. All mice will be humanely killed at the end of our experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexity of the immune system has not yet been reproduced in the lab. Thus all new vaccines and immune therapies require some testing in animals. We do use human cells where possible, but it is not possible predict all the responses to a vaccine, including unforeseen side effects, this way.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use statistics to predict how many mice we need to use to compare two treatments to be certain that we will know if one is better at the end of the experiment. Usually we compare groups of 5-10 mice given different treatments.
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 appropriate species because we have reagents to analyse the mouse immune system and we can model some human diseases. We have designed the experiments to minimise pain/distress/suffering by developing clear concepts of adverse effects that might be seen, based on previous work, and having well-defined humane endpoints

<table>
<tr><td>Project 31</td><td>Murine models of innate and adaptive immunity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunity/autoimmunity/bacteria/sepsis</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>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>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This is an immunology research project looking at immunity in the context of (a) bacterial infections and (b) autoimmune disease, especially multiple sclerosis. In each case, we wish to link up with our studies of immunity in human patients, defining the target antigens in immunity and the differential immune processes that correlate with and explain different disease outcomes.</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>We are trying to advance basic understanding about the nature of immunological mechanism in autoimmunity and infection in a manner that we anticipate and hope will feed into many other studies internationally. In the past these approaches by our lab have informed vaccine development for bacterial infections and the design of therapeutics for human autoimmune disease. Thus, it is hoped that similar benefits may result from the studies proposed here.</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. Used over a period of 5 years Approximately 3000 mice may be bred during this period and of these, up to 500 per year may be utilised in infection, immunisation or disease studies
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?	• Basic immunology immunisation studies are very rarely associated with AE except for the very rare occurrence of inflammatory responses at injection sites. • Infection with bacterial pathogens may, in many cases, depending on inoculation dose and bacterial strain, result in symptoms of bacterial sepsis. This application puts in place humane endpoints such that disease is not permitted to progress to a state that would prolong any suffering caused by this pathology. • Autoimmune disease protocols designed to mimic human diseases such as multiple sclerosis will, by definition, encompass clinical disease phenotypes. The spontaneous multiple sclerosis model described here shows a degree of paralysis developing in a significant minority of mice during adulthood. This PPL describes strict monitoring, time-limits allowed at given disease scores, humane endpoints and measures of additional support for mice showing disease symptoms. In general, every effort will be made to maintain the ongoing colony so as to minimise the number of mice displaying overt disease, except when required for defined studies. At the end of studies mice will be killed by a Schedule 1 method. On rare occasions we may be asked by collaborating labs to supply to them transgenic lines that have been bred under the protocols described in the PPL. In this event, we will formally seek the permission of the HOI.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	Much of our research direction is rooted in our attempt to illuminate complex clinical observations made in human patients about multiple immune pathways, cell-types and, indeed, the interactions of the immune system with other systems such as the

use non-animal alternatives	CNS or the endocrine system. Thus, it is inherent in our approach that work is wherever possible done in vitro with human cells, but this is complemented by studies using mouse models. The direct answer to the question “why can this not be achieved without using animals” is thus that a part of our work towards our biomedical research goals necessitates the study of systemic interactions that lead to disease at the level of the whole animal. In the longer term, a major research aim of our work is to generate large antigen/epitope discovery datasets for the Immune Epitope Database (www.iedb.org). This is a major international effort, funded by the US NIH-NIAID, with the aim of building the immune epitope dataset that underpins the refinement of *in silico* immune prediction software. The tools being generated through this approach are already having massive impact on the replacement of animal work on immune mapping by *in silico* prediction.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We only work with inbred mice and design all of our studies for maximised uniformity of treatment groups (matching for age and sex) so as to minimise experimental variability and minimise group size. We take statistical advice on the design of our studies. We have found that, in terms of T cell specificity and phenotype experiments, we can achieve statistically significant differences between groups with a sample size of 4-6 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.	Our models use inbred, transgenic or knockout mice to investigate immune phenotypes in the context either of autoimmune disease or of bacterial infection. In both of these contexts the mouse models are tractable enough in terms of the ability to be bred and housed, with a well-developed arsenal of genomic, molecular and immunological reagents to facilitate analysis to make them a valid model for understanding multi-system interactions in human disease. The details of immune system development and function are sufficiently similar between humans and mice to make the specific

	pathways and information transferable between the two. In our spontaneous MS models in particular, we have been impressed by the extent to which the murine work faithfully reiterates the immunology and neuropathology of human disease. Our protocol seeks to minimise the number of days during which any mouse will be left with overt autoimmune clinical symptoms to the absolute minimum that is compatible with analysis of the phenotype. We describe detailed measures put in place during this period for increased monitoring and support, including special arrangements for feeding. This PPL contains two severe protocols, to model spontaneous and induced autoimmune disease respectively. The mouse models of multiple sclerosis that we have developed are considered to be among the most faithful to the human disease and have been valuable in the elucidation of pathways and therapeutic strategies, so contributing to the alleviation of human suffering from a devastating disease. We believe that, with the protocol carefully monitored and with mouse numbers minimised, the benefit of using these models may be seen to counterbalance the severity of the protocols.

Project 32	**Service licence for Antibody Production**	
Key Words (max. 5 words)	Service Licence, Antibody production	
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)	**Summary:** The purpose of this licence is to provide a central facility for the production of high quality, non-commercially available polyclonal and monoclonal antibodies for use in scientific studies. **Scientific background:** Antibodies are made by the body as a defence mechanism against foreign material. They circulate throughout the blood and lymph and attach themselves to this foreign material. In this way they help to protect animals and humans from disease. Scientists can make use of this natural ability by challenging animals with very specific foreign proteins thereby causing the animals to create antibodies to these specific proteins. This has revolutionised biomedical research, providing scientists with clinical agents and reagents for scientific investigation. It is important that these important scientific tools can be raised in a competent and ethical manner. This licence aims to provide a service licence under which the scientific community can create antibodies that are not available commercially without the need to raise a large number of individual licences.	

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)?	**Clinical need:** The types of antibodies that scientist require are very variable and often very specific to their work. Such antibodies are rarely available from commercial suppliers. It is important that scientific investigators have access to a supply of individually tailored antibodies and since the completion of the human genome project the demand for such antibodies has increased. As antibodies are so commonly used in research there is a need for them to be made efficiently and in a way that is least harmful to the animals involved. Producing antibodies under this licence allows this to happen in a welfare friendly manner. We feel that the centralisation of the service has benefited both science and the animals. All animal work is carried out by technical staff working to minimum severity protocols and currently work is also carried out to ISO (quality control) standards. We feel that this benefits the clients, the science and the animals, in terms of time, efficiency of procedures and sympathetic handling. We feel that this system provides the best animal welfare standards with the lowest animal usage. The antibodies produced under this licence have a wide range of potential applications ranging from furthering basic understanding biological processes in a specific research programme, through to offering bespoke reagents for use in disease diagnosis and/or therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	Adult rabbits 100 over 5yr project Adult rats 300 Adult mice 1000 Adult genetically modified mice 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?	Injecting animals can cause reaction in the tissue injected. This can be minimised by using good technique and sterile equipment. Blood sampling can cause haemorrhage although this is easily controlled with pressure. Damaged tissue will not be used for sampling again until healed. Veterinary will be sought about any animals which show adverse reactions which are more than minimal. Should the reactions be severe or prolonged the animals will be culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although it is possible to conduct immunisation schedules without animals the techniques available are unreliable. As a result it is only possible to acquire useful antibodies by immunising animals by injecting then with a suitable antigen.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We constantly review animal usage with a view to minimisation, and generally no more than two rabbits/rats or four mice are used for the production of each antibody, allowing for individual variation, unexpected mortality etc. Increasingly, in the case of rabbits and rats, only one animal is required per 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.	Rabbits are traditionally used for the production of polyclonal antibodies although they are increasingly replaced with rats for this purpose. Rats are easier to keep and in most cases produce sufficient serum for the client's needs. Mice are used primarily for the creation of hybridomas for monoclonal antibody production. This is the only species for which can currently be used for this purpose owing to the availability of cell lines for immortalisation. The animals are cared for by experienced qualified animal technicians and scientific procedures conducted by trained and experienced licences. Animals are group housed and provided with environmental enrichment.

Project 33	**Antibody production to Novel Antigens**	
Key Words (max. 5 words)	Monoclonal Antibody	
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
	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
	X	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)	Antibodies are proteins produced by the body to defend against disease by binding to harmful molecules and bacteria. They can be isolated and have become essential tools in a wide variety of biological assays including the diagnosis of disease, roadside testing and the detection of harmful substances. We propose to raise antibodies to various pesticides, drugs of abuse, poisons and other harmful contaminants. Our final patented rapid assays can then be used for screening our environment for harmful contaminants. The main rapid assay that we plan to develop is a dipstick. These types of assays are used eveiy day, the best example is the pregnancy test. They use antibodies raised against the hormones that are increased during pregnancy and if present a blue line is seen. Once the antibody has been raised. Spleen cells are	

	removed and manipulated in culture and fused with a myeloma cell line to produce a cell that will grow in tissue culture and produce antibodies. If successful will result in a permanent source of antibody without the need for further animals.
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)?	Two important areas in which we plan to use them include: Driving under the influence of drugs is an issue of growing concern and has become more prominent than drunk driving in western countries. By producing antibodies to these drugs we can develop dipstick assays that can be used by Police Officers and customs officials to check for the presence of the drugs. When fully developed these assays could be used at the roadside in the same way as the current Breathalyzer. Herbicides and pesticides, whilst beneficial for crop yields, can be very toxic if they are over used and enter our food chains and water courses. Antibodies raised against these pesticides and herbicides can also be incorporated into dipstick assays. They can then be used to check streams flowing into reservoirs for contamination, or contaminants in raw materials prior to food production.
What species and approximate numbers of animals do you expect to use over what period of time?	For our purposes we use, female mice, hamsters or rats usually 6-8 weeks old. Females across all species are better antibody producers. Each project would normally require either the use of 5 mice or 2 rats or 4 hamsters. The projected number for the duration of a five year program would be 1,000 mice, 250 rats, 100 hamsters.
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? (Part 1)	The protocol allows an initial immunization in Freund's. Complete Adjuvant followed by up to 10 boosts in Freund's. Incomplete Adjuvant with a test bleed after 4 boosts. This will enable us to see how the immunization is going and if the antibodies are present and in high enough numbers (high titer). The mouse will be humanely killed and we will remove the spleen and perform the tissue culture. With the number of boosts being a possible 10 I have classed this as a moderate severity and although most of the Projects will come to a conclusion after 4 boosts there is still the potential that some will need more than 4 boosts. Discrete lumps may from time to time develop at the

	site of injection (due to the use of Adjuvants). Although we have not experienced this during the life of the previous license. We still minimise the occurrence by: 3. Restricting the use of Freund's Complete Adjuvant to the primary injection only 4. Distributing the immunogen to at least two sites and not exceeding 0.OSml (0.lml in total) in each site in mice rats and hamsters. In the event of ulceration or an abscess occurring, the NVS will be consulted. If there is no improvement within 2 days the animal will be humanely killed. An adjuvant (a chemical to stimulate the immune response) is co-injected with the harmful molecule. Although these harmful molecules can be toxic in the environment they are used in this context in a dose that is non-harmful to the animals. The adjuvant itself, may cause abscess or ulcer formation. Less irritant alternative adjuvants are constantly being sought; these will be employed as they are developed.
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? (Part 2)	Hypersensitivity may occur and an animal may suffer an anaphylactic reaction. There was a single incidence of this during the previous project license where the immunized animal died after the final immunization prior to the animal being humanely killed and spleen removed. As with any project concerned with raising antibodies to antigens there may be occasions when this occurs. In our experience this is most commonly associated with administration of the final boost of antigen. This final boost is normally an I.V injection of antigen. The incidence of anaphylaxis (an allergic reaction) can be reduced by giving the animal an injection of "Piriton" (anti histamine) immediately prior to administration of antigen. We have reduced the incidence of this adverse effect further by • Omitting administration of the final boost to animals

	which have developed a high antibody titer. • Administering the final boost of antigen by subcutaneous injection rather than intravenously. Since the introduction of these changes there has been no further incidence.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Other technologies to make monoclonal antibodies do exist but are still in their infancy e.g. Phage Display these are quite often weak affinities compared to the high affinities we get when we use conventional immunizations, that utilize the animal's immune system to make antibodies to molecules of interest, Until other technologies are able to provide us with antibodies that have the same affinities and can be grown to the required amounts in tissue culture, animals and hybridoma fusion will be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Not everyone who is immunized will produce antibodies this is the same when using animals. We have increased the changes of successful outcome by using; • Females • Our tailored immunization. • Increased number of boosts for the more difficult molecules. All of these mean fewer repeats using new animals.
3. Refinement Explain the choice of species and why the animal model(s)	Our work has been refined using experiences gained form previous projects. • We use the female of the species generally 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.	females of different species are better at producing antibodies. • Our conjugates are made to insure the best chances of a successful immunisation, minimising the need to repeat with new animals. • All the materials selected are checked to minimise harm to the animal. • We do not use harsh chemicals. • We inject smallest amount possible to minimize lumps, sores etc. Distributing the immunogen to at least two sites and not exceeding O.05m1 (0.1 ml in total) in each site in mice rats and hamsters • All immunisations are carried out by trained animal technicians. • Our immunisation schedule has a test bleeds after the 4th boost to assess the antibodies • All animals with high titers after the 4th boost will be humanly killed and their spleens removed for tissue culture

<table>
<tr><td>Project 34</td><td colspan="3">Immunoregulation during parasitic helminth infection</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Parasites, Immunity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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></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>
</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">The project is designed to define the underlying reasons for resistance to infection by helminth (worm) parasites. Also, because such infections are extremely common and tend to be long lived in humans and animals across the world, we also wish to understand why our defence system does not operate efficiently against them. Not only will the work increase our basic understanding of how the immune systems works against these large infectious agents but, in humans, these kinds of infection are regarded as Neglected Tropical Diseases and, therefore, address clear global clinical needs.</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 project will generate a deeper understanding of how the immune system works following infection by worm parasites. Relatively little is known as to the ways in which the body protects itself following this kind of infection and such knowledge is key to development of new approaches of control such as</td></tr>
</table>

	vaccines. The project will have application to both human and animal health as worm infections are prevalent throughout the animal kingdom and responsible for considerable ill health, in children, in domestic stock and in companion animals across the world.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice are the main species to be studied in conjunction with parasites that naturally infect rodents in the wild. We anticipate using approximately 15,000 mice over a five-year period. A smaller number of rats (up to 500) will also be used over a 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 great majority of treatments will be of mild severity such as general discomfort following infection or transient irritation during an injection or immunisation “vaccination”. In this case the animals resume normal activity almost immediately. Occasionally, the level of severity may increase transiently (overnight) to moderate, with some of the parasite infections used, which is usually associated with the immune system controlling the infection. In these cases the animals may appear “quiet”, show reduce activity and reduced feeding for a short while.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The body’s immune system operates as a co-ordinated response involving multiple cells and molecules at a variety of body sites. Thus, at present, isolated cell cultures or computer modelling cannot accurately model the immune response. Also, no worm parasites can complete their life cycle outside their host. In order to study the immune response to worm parasites in depth, animals are required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use appropriate statistical expertise to ensure that we design experiments using the minimum numbers of animals required to generate meaningful results. We maximize the information gained from each individual animal through the use of the most advanced technologies enabling

	extensive analysis of cells and molecules. We will also develop new cell tissue culture methods to complement our animal studies wherever 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.	In order to study the mammalian immune system we use the mouse as a model system as it is the best understood animal in terms of how the immune systems works with remarkable similarity to other mammals including man. The mouse system also provides us with the most tools to precisely define immune responses thus ensuring clearly defined informative objectives to be met. Moreover, we primarily study parasites that are naturally found in the mouse to ensure that we are investigating the most natural system of host/parasite interactions. As such, parasitic infection by worms tends not to induce severe illness as a general feature. Nevertheless sometimes infection and associated studies can be associated with ill health. Animals under our care are monitored daily by trained technicians who raise any concerns about animal welfare with us and the named veterinarian who decides on the course of action to be undertaken such as treatment. Our experiments are designed with clearly defined endpoints with animal welfare paramount balanced with scientific information gained.

Project 35	**Immunopathology and Immunotherapy of Hepatitis B Virus Infection**	
Key Words (max. 5 words)	Chronic hepatitis B virus infection, T cell therapy, Liver fibrosis, NK cell modulation, Hepatic tolerance induction	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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) (Part 1)	Our research is focused on understanding why immune responses fail during chronic Hepatitis B virus (HBV) Infection. HBV is a major pathogen chronically infecting an estimated 350 million people worldwide, despite the existence of a preventative vaccine. Chronic hepatitis B (CHB) causes severe morbidity due to complications such as liver cirrhosis and liver cancer, leading to 600,000 deaths annually (as reported by the WHO). So far treatments can suppress viral replication but only rarely achieve complete clearance of infection. There is also a risk of viral resistance and toxicity during long- term treatment. Furthermore, antiviral treatment is expensive, limiting its availability in less developed countries, which have the highest rates of infection. An ideal alternative would be an immunotherapeutic approach because the adult immune system has the capacity to control HBV infection.	

	Our aim is therefore to activate the immune system to achieve complete viral clearance. Through our very strong research background and achievements in HBV immunology we are in a unique position to dissect the anti-viral immune response and explore new therapeutic approaches. We will now complement our ongoing work using patient samples with in vivo murine studies. Research on HBV has always been difficult, due to the specificity of the virus for human liver cells (hepatocytes). Although transgenic mouse models, which constantly replicate HBV in their hepatocytes have been used in the past these models are not ideal, since HBV is not seen by the animals immune system as foreign and does therefore not elicit an immune response.
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed) (Part 2)	Therefore, we will make use of a recently described strategy allowing infection of mice with HBV by using the coat of an unrelated virus to deliver HBV into mouse hepatocytes *(3).* CHB infection of these mice is accompanied by an ineffective immune response, similar to the one seen in patients. This model will make it possible to address our 3 aims: 1. To investigate the role of the immunosuppressive liver environment on the anti-viral immune response. 2. To dissect the function of natural killer (NK) cells, which our human work suggests could be beneficial in limiting liver fibrosis, but also harmful as NK cells can kill T cells, which are critical to combat the virus. 3. To modulate T cell anti-viral immunity, by engineering T cells with novel properties to test their therapeutic efficacy.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work proposed will enhance our understanding of the immune response to CHB and is an essential step towards the development of targeted immunotherapy of this infection and related conditions, It will also provide new insights into the influence of the liver environment on immune responses in general.

	The proposed work will further be of relevance to other diseases characterised by chronic inflammation and progressive fibrosis, in particular a major cause of morbidity and mortality in HIV patients co-infected with HBV/HCV and in patients with alcoholic and nonalcoholic liver disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, approximately 800 animals over the period of the 5-year grant, reflecting the work of 2 postdoctoral researchers
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?	Neither acute nor chronic HBV infection have been reported to lead to any severe suffering, the expected level of severity is mild to moderate. All animals utilised for our research will be culled at the end of the experiments, to study specific cells and tissue.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Access to relevant human tissue, such as the HBVinfected liver is limited. We are not able to investigate the human immune response in vivo and using human cells in culture limits our understanding of the interplay of the components of the immune system. We will use the proposed animal model to investigate the relationship of the different immune components in vivo by studying the liver, spleen and other tissues directly. The mouse model will also allow extensive functional analysis of specialised liver-derived antigen presenting cells. We will assess the dominant in vivo effect of NK cells (for which we have identified several potential roles from our human studies in culture). Importantly, the animal model will allow us to test and refine therapeutic interventions. In particular, we will test the efficacy of different molecular modifications of engineered T cells in vivo as a prelude to future clinical translation.
2. Reduction Explain how you will assure the	The bulk of this study will be conducted using human samples, making up about 60% of our work and only about 40% will utilised the animal model.

use of minimum numbers of animals	Whenever possible in vitro studies using human or murine tissue will be used. New therapeutic strategies will be tested in animals only if we have an indication of their usefulness from our human work. The number of animals planned reflects the work of at least 2 postdocs. Since the projects of the two postdocs are linked we will utilise the same animals whenever possible, e.g. to study two different immune cells purified from the same mouse. Control animals might also serve to control for two different experimental questions and the postdocs will work together closely. We will keep up to date with current developments in our field in order to refine techniques, reduce animal usage and avoid unnecessary duplication of published experiments. By staining for 10-12 different immune markers in each sample we will maximise the information gained from a minimum of samples. We can also indirectly measure liver damage and HBV activity using serum ALT and animals and why you cannot use non-animal alternatives researchers HBV serology. These measures will allow us to monitor infection and immune responses using venesection, thereby substantially reducing the overall number of animals used. Careful consideration will be given to the number of animals needed to give meaningful results in consultation with statisticians at UCL to optimise 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.	Ducks and Woodchucks can be infected with a hepatitis virus similar to human HBV, however, their use is limited due to their outbred nature and their immune markers being ill-defined. Mice are well studied and their immune components well defined, but they are not natural hosts for HBV. A novel route of packaging HBV into the coating of an unrelated virus, which targets the murine liver, now allows us to chronically infect mice by injecting a well tolerated volume of packaged HBV virus. This model can be adjusted to either achieve an acute or chronic infection, allowing us to dissect the

	mechanisms leading to the two different outcomes. Neither acute nor chronic HBV infection have been reported to lead to any severe suffering of their murine host. Animals will be kept in well-maintained housing, which will also reduce experimental variability caused by environmental stresses. In the event of unexpected adverse effects we will use humane endpoints.

Project 36	**Production and function of blood cells**	
Key Words (max. 5 words)	Red blood cell, T-cell, thymus, immunity, anaemia	
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 project aims to understand how white blood cells are produced in the thymus and bone marrow and how red blood cells are made in the bone marrow and spleen. We aim to test the function of these cells if we change some molecules that influence their production. We also aim to test what happens to white blood cells when a thymus is transplanted into mice, which do not have a thymus.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Understanding how white blood cells are made and how they function is important for all disease involving the immune system (infectious disease, autoimmune disease, leukaemia). Understanding how red blood cells are made is relevant to all human disease which influences red blood cells (anaemia, malaria, blood cancers).	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice Over 5 years we will expect to use up to 10000 animals. Most of these will not be experimented on them selves, but will be involved in the breeding programme and as a source of tissues.	
In the context of what you	Nearly all experiments in this project will be mild and	

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?	not have adverse effects on the animals, and the animals will at the end be humanely killed or transferred to another licence for breeding. In very few experiments (<100 mice over 5 years) the adverse effects will be moderate. In these cases the animals may lose weight and loss of appetite over a period of 7-10 days, after which they will recover or 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 majority of experiments will be carried, using mice as a source of tissue only. Because the mice used will be GM, as this is the only current reliable way to delete/mutate a gene from a given tissue, the breeding of the animals will be a regulated procedure. We will minimise the number of animals used by where possible harvesting multiple tissues from animals sacrificed and so using any individual mouse to enable us to do a range of experiments in parallel. We will carry out the minimum number of experiments to allow us to obtain statistically significant results. To minimise the number of animals used, it is important for experiments to be designed well and to include the appropriate controls and that experimental groups are of sufficient size for statistical analysis.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The production of blood cells and the development of the immune system and the activation of the immune response are dynamic and tightly regulated processes that involve many different cell types, and so cannot be studied in cell lines. Where possible, however, we will use organ and cell culture techniques to minimise experiments on live animals. To investigate some processes which involve migration of cells from one tissue to another in an animal or involve a systemic response the animal involving multiple tissues, it is necessary to carry out the experiments on a live animal, as organ culture systems would not suffice. In addition, we will have to breed GM animals as a source of tissue for experiments because genetic modification (transgenesis and knock-out technology) are the only available means of reliably mutating a gene in a

	developing tissue.
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 appropriate species because technologies for genetic modification are well established in mice and because the GM mice necessary for this study have already been produced. Mice are also the most established model for the study of immunity and haematopoiesis. Suffering will be minimised by maintaining immunodeficient mice in individually-ventilated cages to reduce the risks of infection; by careful monitoring of animals after procedures; by monitoring mice for any signs of ill-health (e.g. Ruffled-fur, changes in posture, reluctance to feed, weight-loss); by appropriate use of anaesthesia and analgaesia; by culling mice that are deemed to be sufferinq (as judqed by chanqes listed above).

<table>
<tr><td>Project 37</td><td colspan="3">Investigating the immune response in the oral cavity</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Immunity, inflammation, infection, oral</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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>
</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">Dental diseases inflict a significant health and socio-economic burden. The estimated cost of NHS funded dental treatment in Scotland is between £350 and £500 million per year. The main diseases of the oral cavity are periodontitis (gum disease) and dental caries (tooth decay). Although not direct causes of mortality, these diseases are major public health concerns because they affect the majority of the population and have a negative impact on oral health, ability to chew, appearance, quality of life, dental care costs and tooth loss. Both periodontitis and caries are the result of a breakdown in the balance between the body’s defenses (the immune system) and the bacteria in the mouth. Several studies have shown that periodontitis is associated with poor general health, in particular heart disease, diabetes and rheumatoid arthritis. Inflammation caused by periodontitis may be responsible for its association with poor general health. Currently, this relationship is poorly understood. This work aims to gain information on the inflammation initiated by periodontitis, identifying potential ways that oral disease impacts on general health, and to look for new treatments for oral disease.</td></tr>
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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 primary goal is better understanding of the immune response in periodontitis, and a better understanding of how the immune response in the mouth may impact on general health. The work offers secondary potential benefits in developing new screening tools, preventative measures and treatments for oral disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse 4600 over 5 years. The majority of these will be from breeding genetically-altered animals. We plan to only use mice (rather than other rodents) as the mouse model is well accepted and the availability of genetically-altered strains will help answer important questions. We will minimise the use of animals by seeking alternatives (such as laboratory based cell culture methods) wherever possible, and through careful experiment design.
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 with experimental periodontal disease do not show any adverse effects (rather like humans – in whom the disease is 'silent' and painless). Animals with experimental arthritis show footpad swelling. We employ a mild model of arthritis and the swelling is limited to one foot and lasts for a few days only. Animals are monitored regularly and the experiment terminated if footpad swelling or any other lesion develops beyond a defined limit, or if mice show severe lameness or signs of distress. Certain genetic alterations to components of the immune system render animals more susceptible to infection. Where necessary, immune-deficient animals are maintained in filter cages to prevent infection, and any affected animal will be removed from the experiment or receive veterinary attention. Some procedures require the animals to remain still and are therefore performed under anaesthetic. The level of anaesthesia will be maintained at sufficient depth for the animal to feel no pain, minimizing adverse effects by ensuring accurate dosing.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We strive to use cell cultures rather than whole animals wherever possible. However, the complexity of the host-bacteria interaction means these cell culture approaches cannot currently adequately reflect the complexity of the system in a living animal, which is required to answer our research questions. We work with and continue to develop our cell culture systems, aiming to aid in the replacement and reduction of animals in studying the immunology of infection.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Based on our previous data, we have liasied with a Statistics professional to ensure that in all experiments, group sizes will reflect the minimum number of animals required to perform proper statistical analysis. Where we do not have sufficient data available to make reasonable predictions, we will run smaller pilot studies. Where possible, we coordinate the timing of experiments such that an identical control group (for example uninfected, untreated) can be used for more than one experiment. This is planned with regular and careful discussion of all those working on the licence. Moreover, there is careful communication to ensure that experiments are not repeated if the tissue/cell type of interest is available from another similar 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.	Mice are the lowest vertebrate group in which the immunological models have been developed. The mouse immune response to bacteria is commonly used to model human immune response to bacteria, and to investigate novel therapeutics. The mouse models of periodontal disease and arthritis that we propose to use are well documented and accepted by peer-review. These are accepted approaches with minimal adverse effects in the mouse. The experimental arthritis models will be the least severe available to answer the question. All mice are carefully monitored and advice sought where necessary.

<table>
<tr><td>Project 38</td><td>Using zebrafish to understand inflammation resolution.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Neutrophil, macrophage, inflammation, innate immunity, zebrafish</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>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>Yes</td><td></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>Failure of inflammation resolution underpins a wide range of diseases, of major importance to health. The neutrophil is a key driver of tissue damage in chronic inflammation, yet there are no therapeutics in clinical use which overcome persisting neutrophilic inflammation to drive resolution and healing. Work from my group and others has shown that inflammation resolution can occur by regulated migration of neutrophils away from inflammatory sites and that this can be blocked by pro-inflammatory stimuli. I therefore hypothesise that neutrophils are retained at inflammatory sites by “retention signals” that delay reverse migration and so prevent inflammation resolution. In this programme of work, I will identify the nature of retention signals and how these overlap with survival signals, then explore the intracellular signalling consequences of retention signals, looking for druggable targets with therapeutic</td></tr>
</table>

	potential. While targeting neutrophil recruitment leaves tissues unprotected against infection, targeting retention signalling by contrast allows removal of unwanted neutrophils without impacting host defence. Such pro-resolution therapies would revolutionise treatment of many of today's most important diseases.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project seeks to better understand how inflammation resolves. We hope that this would lead to increased understanding and in due course new treatment approaches for inflammatory disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mutant and transgenic fish are generated, which are used to generate progeny, used almost exclusively for unregulated procedures. These experiments will involve imaging inflammation in realtime. In order to see how the host immune system functions, a fully constituted immune system is required. This cannot be established in vitro. The proposed study uses larval zebrafish (*Danio rerio*) rather than adult mice. Human primary cells are used wherever possible to avoid animal use. The number of zebrafish used is determined primarily by the number of breeding adults required to supply the unprotected larvae for the studies suggested. 102,500 adults will be required over the course of the 5 year programme of work, but these will all be healthy, and used for mating purposes only – no suffering is anticipated.
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?	Some adults may need to have gametes manually expressed. This extends their reproductive health and reduces the number of fish required overall. Some fish may have the tailfin clipped under anaesthetic to determine their genetic identity. Both these latter procedures are performed under a brief immersion anaesthetic and cause no lasting harm to most fish. Finally, up to 2,500 larvae up to 10 days post fertilisation will be studied where appropriate experiments cannot be performed on

	larvae before the onset of independent feeding. Zebrafish were chosen because of the near transparency of the larvae, coupled with the ready availability of transgenic and mutant resources make them the only model organism in which the individual host cells can be visualised interacting with pathogens in vivo, and in which the effects of genetic manipulations can be readily observed. Animals will be sacrificed before they suffer disease at the end of their natural lifespan, or earlier if indicated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation is a complex process requiring interaction of multiple cell types and cannot meaningfully be modelled in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Most experiments are performed on larval zebrafish. The mature fish in this programme are used to generate larvae only. We have extensive experience of the assays used, and are confident of our calculcations of the minimum number of fish 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.	Zebrafish are the model with the lowest neurophysiological sensitivity suitable for such studies (a vertebrate immune system is sufficiently similar to humans to be useful, but insect or worm immune systems are not). This model has minimal impact on animal welfare.

<table>
<tr><td>Project 39</td><td>Initiation and maintenance of immune responses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immune response, vaccination, autoimmunity, tolerance</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5years</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>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>In order to fight diseases without generating too much collateral damage the immune system has to make careful and balanced decision about what to attack and what not. A wrong decision has disastrous effects. A failure to respond to a dangerous pathogen can result in raging infections and ultimately death. On the other hand, inappropriate responses can lead to autoimmunity, allergies and in the case of pregnant women to miscarriages. The decision whether to launch a response or not is made by three cell types. Antigen presenting cells present fragments of possible pathogens to pro-inflammatory cells and anti-inflammatory cells. The pro-inflammatory cells try to start immune responses whereas the ant-inflammatory cells try to prevent them. We want to understand how these cells ‘talk’ to each other. This may allow us to develop therapies for autoimmune diseases, better vaccinations and to induce immune</td></tr>
</table>

	responses directed against cancers.
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 understanding of the immune system. Our long term aim is to modulate the immune response to improve vaccinations and to prevent/treat undesirable immune responses (autoimmunity, graft rejection). **2. Translational benefits** A better understanding of these mechanisms will allow us and the scientific community to develop novel approaches to modulate immune responses. We actively dedicate part of our time on research on early translational aspects. For example, in the past we have developed a method that allowed us to stop arthritis in a mouse model. **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 vaccination improvement, cancer treatment, the prevention and treatment of autoimmunity, management of transplant rejection.
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 in *vitro/ex vivo* experiments require cells from donor mice. We expect that the research programme will require approximately 35500 mice over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Our experiments in mice will mimic the various scenarios the immune system (vaccination, infection, autoimmune tolerance, anti tumour response) has to deal with in an experimentally controlled fashion. The majority of mice are expected to experience only very mild, if any signs of discomfort. However, as we want to understand how the immune system fails and how to avoid this, some animals will have 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). 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 appropriated 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 have 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 animal 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. We are actively involved in researching

	novel approaches to improve the 3Rs. For example, we are exploring new microfluidics methodology which not only allows us to reduce the number of primary cells and thus number of donor mice used in the experiments, but also might be used to replace some *in vivo* approaches in the future.
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 chosen because they have been shown to yield reproducible results. Mice are the lowest species in which these experiments can be performed. As many aspects of the immune system of mice are already well understood, the reagents available are usually of high quality. This makes the data obtained in the experiments more reliable, which helps to reduce the number of animals. 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 breeding programmes means that we minimise the numbers of animals produced that are surplus, 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.

<table>
<tr><td>Project 40</td><td colspan="3">Methods to enhance or suppress immune responses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Vaccine, autoimmune disease, cancer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3"></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>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></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 colspan="3">The objectives of the project are to refine and optimise methods we have identified (1) to enhance immune responses to vaccines, and (2) to specifically reduce immune responses</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">Vaccines against cancer, to be given as treatments, are just coming into use and might form an important part of the physicians’ armoury in the future, but a major block is generating a strong enough immune response. On the other hand autoimmune diseases such as rheumatoid arthritis and multiple sclerosis are caused by strong immune responses to self molecules. If we can demonstrate our methods are able to strongly enhance, or suppress immune responses, then these methods could eventually be applied to cancer patients and possibly patients with autoimmune diseases and could have dramatic effects on illness and death</td></tr>
<tr><td>What species and</td><td colspan="3">Mice only. We estimate 720 per year over the</td></tr>
</table>

approximate numbers of animals do you expect to use over what period of time?	immune enhancement (cancer vaccine) and the immune suppression (autoimmune disease) work.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The vast majority of the animals will simply be immunised and have blood taken, so will have only mild adverse effects. Some mice will be given tumours but even these will be culled before the tumours cause more than mild to moderate effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We must measure specific immune responses, that is, those which are directed at particular host molecules. Specific immune responses are the result of interactions between very rare (one in a million) cells called B cells with very rare (also around one in a million) along with specialised cells called antigen presenting cells. These interactions take place within tissues which have an architecture designed to allow these unlikely meetings to occur. The response to vaccines or to other agents cannpt be mimicked in culture systems at the moment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Numbers used will be as low as possible to allow meaningful data to be obtained as determined by statistical analysis. Wherever possible multiple immunological measurements will be obtained from the same animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the animals of lowest sentience with immune systems extremely similar to those of humans. . No cancer vaccine will be tested in a cancer model without having first shown in can induce good immune responses. Cancer models are designed such that they are over before the mice suffer any serious effects.

<table>
<tr><td>Project 41</td><td colspan="3">Immunomodulation by helminth parasites</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Parasite, immunology, immunomodulation, therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></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 colspan="3">We are investigating molecules produced by helminth (worm) parasites which control the immune response in order to address the growing need for new anti-inflammatory drugs. Autoimmune diseases are on the rise in industrial countries and treatment options are poor and expensive, as is treatment for chronic gut inflammation and chronic obstructive pulmonary disease. Parasitic helminths offer a new way of treating those diseases and there have been promising clinical trials with live helminth infection. However, live infection carries considerable risks to health, and thus there is a need to understand how parasites regulate the immune system. We aim to isolate, characterise and utilise defined molecules which mimic these immunoregulatory mechanisms in order to develop new drugs which can ultimately be used to treat human diseases. The knowledge gained will also enable us to identify vaccine targets which elicit protective immune responses against those</td></tr>
</table>

	helminths that pose a disease threat to humans. To generate parasite material and to determine how new avenues of immunoregulation can be used in the safest and most beneficial way for humans, we need to make use of established animal disease models in which we can isolate individual cell populations, transfer cell populations and use genetically modified animals missing specific cells or genes to ensure efficacy and safety. Many experiments will involve bioinformatic (at the computer) and in vitro analysis. Only when we need to model inflammation and analyse cell interactions too complicated to mimic *in vitro* will we use animals. We also need rodents as hosts for the parasites in order to isolate secreted molecules. The least stressful techniques yielding the most comparable (to current literature and human data) and reliable results with the lowest numbers of animals to gain statistically significant and convincing data will be used throughout the project.
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 aims to isolate and test molecules which have potential to treat diseases such as arthritis, colitis and asthma, characterised by excessive infiltration of activated white blood cells into joints and the lungs, as well as autoimmune diseases such as multiple sclerosis, type 1 diabetes and lupus. In addition, development of vaccines against worm infection would represent a major advance in controlling a class of pathogens which have a huge impact on global health, greater than HIV or tuberculosis when measured by disability-adjusted life years (DALYs), a time-based measure that combines years of life lost due to premature mortality and years of life lost due to time lived in states of less than full health.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, rats: 5 years gerbils: 1 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?	The procedures we propose are all considered mild, except the model for intestinal inflammation and radiation. Animals will be infected with parasites which, at the doses used, do not have side effects, and they will be humanely sacrificed before parasites or cells are retrieved and analysed. Intestinal inflammation will result in diarrhoea and weight loss but will be closely monitored and procedures will be stopped if animals reach rigorous and well-defined human endpoints. When mice are irradiated and then reconstituted with bone marrow cells, animals will be kept in sterile conditions to prevent infection with pathogens, will get dissolved food and easier access to food and drink and will be monitored every 2 hours the first day and then twice a day until they recover fully. Wherever possible analgesics and/or anaesthesia will be used to reduce side effects or pain. All animals will be humanely killed after experiments or should unexpected and harmful side effects arise.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation is the process in which leukocytes migrate to the site of infection and/or tissue damage. Whilst some aspects of this process can be mimicked in vitro (and will be assayed in the project), the process of extravasation and subsequent migration through extracellular matrices can only be assayed in an in vivo setting. Animal models are also crucial to mimic human disease, as this is not possible in vitro and it would not be ethical to study new drugs or drug targets on humans. Animals also have to be used to provide parasite material for input into in vitro and in vivo assays. When screening for immunomodulators intended to provide a new means of treating human infection, the use of a mouse model for proof of principle is crucial, and provides a plethora of data and well-defined read-outs in relation to pathology and immune responses to ensure convincing and

	corroborated results.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will seek to perform as many experiments in the project as possible without use of animals using cell line, in vitro assays and in silico (at the computer) approaches. Where use of animals is necessary, in order to ensure that high quality, reliable and valid data is extracted from the minimum number of experiments, the ARRIVE guidelines (Kilkenny et al., 2010) will be followed. http://www.nc3rs.org.uk/page.asp?id=1357
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodents are natural hosts for the parasites we study and their immune system is extremely well characterised and closely resembles the human immune system for the diseases we model. Skilled and experienced licensees will undertake all techniques, and sympathetic animal handling, injection and blood sampling techniques will be used throughout in order to minimise discomfort. Lengthy or potentially stressful techniques will be performed under anaesthesia. The animals will be housed in excellent conditions with appropriate bedding and nesting material, and will be monitored and cared for on a daily basis by professionally trained staff. If animals become unexpectedly seriously ill they will be humanely killed.

<table>
<tr><td>Project 42</td><td>Rodent models of Disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Genetically altered, breeding, disease models</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></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>This project will create, breed and maintain rodents (mice and rats) with genetic alterations showing the desired disease phenotype (characteristics) and supply them as either tissue for use in in vitro (isolated tissue) experiments or for transfer to other project licences which require that particular disease phenotype for the scientific research.</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 use of live animals is needed in order to breed strains of mice which mimic and show phenotypes (characteristics) typical of human diseases in order to help develop novel treatments and prevention of disease.

Genetically altered animals provide a method of evaluating the roles of genes in a complex system, building on information that can be obtained in non animal models. Genetically altered animals are more precise in comparison to traditional animal models. Many carry defects in their immune system which allow the grafting of cancer cells for development of new therapies.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>It is anticipated that we may use up to 25,000 mice and 1000 rats per year. These numbers are a reflection of the number of different genetic alterations that are used at this institute. In some cases complex breeding programmes are required where multiple genes may be altered in one animal.</td></tr>
<tr><td>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</td><td>The majority of the mice will not experience any pain suffering or distress as a result of the genetic alteration – a small tissue biopsy may be taken from the ear of an animal but this will cause no more than slight transient pain.</td></tr>
</table>

happen to the animals at the end?	Where animals an impaired immune system they will be maintained in barrier conditions to prevent any infection developing. Some animals will undergo surgery to either transfer embryos into the uterus or to induce sterility in males by a vasectomy– aseptic techniques are used to minimise infection and analgesia (pain relief) is provided. Where a gene is deleted by administration of a compound in the diet e.g. tamoxifen the frequency of monitoring will be increased to ensure the diet is palatable and the welfare following gene deletion is monitored closely. Animals will either be humanely killed for tissue collection or transferred onto a scientific project licence supporting research programmes at the institute.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The most complex interactive processes of living mammals are not reproducible in vitro in the laboratory and the possibility of using non animal alternatives is considered by all research projects before the decision to use animals is taken. Ifthere are no non animal alternatives the use of live animals is needed to understand the role of genes in the development and or treatment of diseases.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Database searches will be made before any new animal model is created to ensure that duplication of models does not occur. The use of tissues from established models will also be considered if appropriate. A centralised service for the breeding ensures that best practises are used and that breeding numbers are controlled to minimise the production of surplus animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rodent models will be used where other lower sentient models are not appropriate to study gene function i.e. zebrafish. The mouse provides an appropriate species as the genome has been sequenced and can be manipulated. There is a high degree of homology with the human genome sequence meaning that the basic biological and pathological processes are

	similar or identical to those in other mammals including man. The majority of methods used will cause minimal pain or suffering Where a minor surgical procedure is required which may cause moderate pain or suffering this will be carried out under general anaesthesia and analgesia (pain relief) under the guidance of the veterinary surgeon to minimise any discomfort to the animal.

<table>
<tr><td>Project 43</td><td>Natural variation in immunity in wild rodents</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunopathology, infection, immunology, ecology, zoonosis</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>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>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td>Yes</td><td></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>The aim of this project is to understand the causes and consequences of variation among individuals in the natural environment in their response to infection.

To achieve our aim we will study wild rodent species that are abundant in the UK, naturally infected with multiple pathogens and for which we have now generated genome sequences and immunological assays to measure key components of the immune response in the natural environment. Wild rodents are thus a model system that will cast light on variation in responses to infectious disease generally.</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 benefits of this research will be to identify the types of individuals, and the environmental circumstances, that make individuals more or less vulnerable to infection and disease. Within medical and veterinary settings it is important to understand why some individuals are predisposed to make immune response that render them more likely to develop disease following infection, or to fail to be protected after vaccination or to develop autoimmune pathologies. Doing so would be an important step in first identifying and then developing treatments to protect them from disease. This project will also help us to understand how to conserve natural populations threatened with disease and how to mitigate against zoonotic infections (infections passed from wildlife</td></tr>
</table>

	populations to humans).
What species and approximate numbers of animals do you expect to use over what period of time?	Field voles 3000 in total over 5 years Bank voles 600 in total over 5 years Wood mice 600 in total 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 will be caught from the wild, identified using transponder tags, information on sex and body condition recorded, and small blood samples taken. This monitoring procedure may be repeated up to every 2 weeks to create a data set for each animal through time. This causes minimal distress to the animals and only in a small number of cases, far less than 1%, do we expect animals to experience any adverse effects such as shock. Following our work, animals will either be culled humanely (in order to obtain tissue samples) or released back to the wild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The response to infection is a complex response involving many tissues and can only be studied in a live animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use powerful statistical and computational approaches that make full use of data collected on each individual and so minimise the total number of animals used. We are supported by leading statisticians and computational biologists. After the first sampling season, we will perform an initial analysis to determine whether numbers can be reduced while retaining statistical rigour.
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.	Wild rodents will be used since these are genetically close to lab mice, and hence we can use many of the resources developed for these, but are a better model to study natural variation in immunity since wild rodents are genetically diverse and exposed to environmental variation. The work will be primarily monitoring and the techniques used will be minimally invasive, well-established and performed by skilled personnel.

Project 44	**Lymphatics and cell trafficking during inflammation**
Key Words (max. 5 words)	Lymphatics, Cell migration, Inflammation
Expected duration of the project (yrs)	
Purpose of the project (as in section 5C(3)	Basic research: Yes ~~No~~ Translational and applied research: Yes ~~No~~ Regulatory use and routine production: ~~Yes~~ No Protection of the natural environment in the interests of the health or welfare of humans or animals: ~~Yes~~ No Preservation of species: ~~Yes~~ No Higher education or training: ~~Yes~~ No Forensic enquiries: ~~Yes~~ No Maintenance of colonies of genetically altered animals: Yes ~~No~~
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To study the changes in lymphatic structures and function during inflammation.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will provide new insight into the involvement of the lymphatic system under inflammatory conditions such as rejection of a transplanted organ or inflammatory bowel disease. Better understanding in this area will aid the development of new treatment for patients suffering from these conditions which are associated with considerable morbidity and mortality.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use mainly mice in this project. In the region of 7,000 over the 5 years of the project. A small number of rats, in the region of 300 will also be used during the same period.
In the context of what you propose to do to the animals,	Adverse effects that may be experienced following the transplantation procedure or colitis induction

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	include: lack of appetite, reduction in body temperature (shivering), hunched posture, reduced mobility and piloerection. Adverse effects are expected to be at a mild to moderate level of severity. At the end of a study, the animals will be killed in a humane fashion.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project is designed to study the mechanism of whole organ transplant injury and bowel inflammation. Therefore, only the use of animals will allow such a study. Initially, before any animals are used, all treatments will first be tested in the laboratory using non-animal methods.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experienced transplantation surgeons achieve good reproducibility of the transplant procedure. Accordingly, we will use small numbers of animals in each experimental group, typically 5-10. For kidney transplants, one donor animal will provide one organ for transplantation into one recipient. Within our department we have a dedicated statistician who provides advice on study design and provides calculations to accurately assess the smallest number of animals required for statistically meaningful results to be achieved.
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 have been chosen because of the number of genetically modified strains available to work with. We have some mice that express the fluorescent protein YFP to allow us to differentiate between donor and recipient lymphatics in the transplantation experiments. This is important because donor lymphatics will appear as "foreign" to the recipient and therefore, function differently to lymphatics from the recipient. Measures are taken to minimize harm to the animals. Firstly, risk of infection in the animals is minimized by applying strict asepsis throughout all procedures. Secondly, animals will be under anaesthesia for relatively short periods of time to

	reduce harm to them. We will avoid any post-operative discomfort to the animals by regular monitoring and providing pain control in the form of analgesia (peri-operative analgesia as advised by the Named Veterinary Surgeon will be used) and keeping animals comfortable in a special warm environment in which the temperature is maintained at 28ºC for 24 hours post-operatively. Animals showing adverse effects may receive supportive care and treatment as advised by the Named Veterinary Surgeon or they will be humanely killed if their condition gives particular cause for concern.

Project 45	**Roles of monocyte/macrophages in metabolic and inflammatory diseases**	
Key Words (max. 5 words)	Immune system, macrophages, monocytes, development, 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
	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)	Monocyte/Macrophages are critical effectors and regulators of the inflammatory response, an ancient mechanism and an important component of the immune defence against microbes and parasites. The anti-microbial role of monocyte/macrophages enables the acute inflammatory response, which ultimately allows for the survival of individuals. However, in the long term, chronic inflammation is also responsible for diseases that decrease the quality of life of adults and ultimately can significantly shorten the lifespan of adults such as in type 2 diabetes or cardiovascular disease. Monocyte/Macrophages have considerable functional diversity at different anatomical locations, as well as within each tissue, and depending on pathophysiological conditions. Because this diversity is not well understood, both the mechanisms that control their functions are not well characterized. Our objectives are to better understand of the	

	development and functions of Monocyte/Macrophages to characterize how they control the inflammatory responses in living organisms and identify mechanisms responsible for inflammatory diseases like type II diabetes, cardiovascular diseases and artheriosclerosis.
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)?	Even with conventional drug development chronic inflammatory diseases represent leading causes of pain and early death in the world. To better translate our scientific knowledge of the roles of monocyte/macrophages into real benefit for human health, we must admit the limitations of this knowledge, and invest on characterizing in details the functions of these cells in vivo, during different developmental stages, under normal physiological conditions, and in conditions that drive diseases. This knowledge will allow us to understand the mechanisms responsible for chronic inflammatory disease such as the complications of obesity, atherogenesis, and autoimmune diseases such as Lupus, and ultimately devise new strategies to prevent disease and/or treat patients efficiently, avoiding side effects.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice (Mus musculus) for our studies, because most of the processes and mechanisms of the immune system seen in humans resemble the murine immune system. Based on our experience and detailed planning of the current project, we anticipate that 15000 mice will be required over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The level of severity of the planned experiments in this project is mild or moderate. All methods are well established in our lab and will be run by trained staff. In any case, one animal is showing a sign of side effects including pain and suffering, we will immediately stop the experiments and/or advice will be taken from the current veterinarian in the facility. All animals undergoing surgery are in deep terminal anaesthesia during the procedure, which is kept and

	maintained for the whole duration of the experiment. Where mice show ill-health or discomfort (e.g. lack of grooming, feeding or normal movement and social interactions in the cage, signs of infection etc) they will be killed by a Schedule 1 method

<table>
<tr><td>Project 46</td><td colspan="3">Murine models of human haematopoiesis, therapy and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Gene therapy, haematopoiesis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</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>
</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">Our aims are to improve our understanding of current primary immunodeficiencies and lysosomal storage diseases to improve treatment. We also aim to develop and improve upon current gene and cellular therapies for these diseases with the aim of increasing the number and types of diseases and number of patients currently treated by gene therapy.</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 potential benefits are better treatments and potential cures for inherited genetic diseases and malignancies through cellular and gene therapy.</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">We will use mice exclusively (approx 8500 over the 5 year project)</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?	Some animals fail to thrive due to the nature of the genetic disease. Some animals may get malignancy due to gene therapy treatments. Our aim is to improve current therapies to avoid this. We expect the severity level to be moderate. All animals are to be culled at the end of procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Currently no other non-animal models exist that mimic the genetic diseases. The complex organisation of the haematopoietic system makes *in vitro* culture unsatisfactory. *In vivo* experiments are required to measure engraftment and longevity of haematopoietic stem cells and development of functional immunity because the complex multifactorial interactions cannot be assessed *in vitro*. In addition we want to assess the impact on health and behaviour. The safety of vectors over long periods of time and the potential for malignancy can only be adequately assessed *in vivo*. In fact the current guide lines state *in vivo* testing is mandatory for testing efficacy of treatment in disease models, and assessing safety for the whole organism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible, animal experiments will be designed based on information gained from studies of immune cells conducted using cell lines, human primary cells or cells isolated from animals humanely killed. The optimisation of vectors in cell lines should reduce numbers and engraftment and efficacy data from one disease can be used for others to avoid unnecessary repetition. Where animals have to be genotyped this will be done as early as possible and wild-type littermates will be used as controls as well as sharing controls for comparable treatments thus minimising the need to purchase animals. Where possible, each animal will be used for multiple analyses post mortem (for example histological and immunological).
3. Refinement	We have chosen mice as we are studying models of inherited disease and mice are the most

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.	appropriate and best characterised lowest vertebrate group animal model. Murine models are regarded as the most appropriate judged by peer reviewed publications. Where possible we propose to treat before the onset of disease with therapies rather than treat once disease has been established to minimise suffering. The therapies we develop in the mouse will be directly transferable to patients avoiding unnecessary replication. We have experience with breeding immunodeficient animals and with long established protocols that have well defined end points so are able to provide high level training and thus competence for experimental procedures.

Project 47	**Mechanisms of cellular guidance in vivo**	
Key Words (max. 5 words)	Leukocyte, neutrophil, chemoattractant, cell migration, chemotaxis	
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)	White blood cells (leukocytes) protect us from infection. To achieve this, leukocytes need to exit the blood stream and then navigate within tissues in order to find and eliminate invading microbes. This crucially depends on their ability to follow guidance cues produced upon infection. Any error in this process can cause inflammatory diseases, such as chronic inflammation, or failure to fight infections. How leukocytes navigate through tissues is not fully understood. To investigate this problem we will use zebrafish, which are simpler than mammals but have a similar immune system and are optically transparent, allowing us to visualise leukocyte movement by microscopy. Using zebrafish, we have so far discovered that one type of guidance cue - small proteins called 'chemokines' - needs to interact with specific sugars in the tissues in order to guide leukocytes to microbes. We will elucidate further the mechanism of action of chemokines. We will investigate how tissue sugars and other tissue components affect chemokines and how leukocytes process this information to get to the site of an infection. We will also examine how other molecules help guide leukocytes to microbes. We thus aim to provide a better	

	understanding of how leukocytes read guidance signals, which could ultimately lead to new treatments for human 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)?	Our research project is of major biomedical importance. Leukocyte migration to sites of injury or infection is essential for defence against invading microbes. Conversely, abnormal leukocyte recruitment can drive chronic inflammatory diseases. For this reason, controlling leukocyte migration is a major biomedical target. This target has yet to be met, as we still have little understanding of how leukocytes are guided within the body. Although many types of guidance cues have been identified over the past decades, their actual mode of action and interpretation by leukocytes are poorly understood. If we can understand this, we can be in a much better position of designing effective and selective treatments for inflammatory diseases. Beyond the immune system, guidance mechanisms are utilised in embryogenesis and wound healing, whereby cells move in order to build or repair tissues, and in cancer metastasis, whereby tumour cells - like leukocytes- disseminate by traversing the blood circulation and invading other tissues. Thus, fundamental knowledge gained from our studies is likely to have broad biomedical implications.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 9600 adult zebra fish for breeding purposes only, 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?	There are no adverse effects expected in relation to breeding the animals or the genetic modifications used. In limited cases (up to 5%) we will need to anaesthetise fish for In Vitro Fertilisation (IVF) or genotyping/phenotyping purposes (up to 40%). The expected level of severity is mild. Fish produced under the authority of this project will either be killed or supplied to other projects with authority to use genetically altered fish of this type.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The key aspect of our programme is to visualise leukocyte behaviours in situ by advanced light microscopy techniques. We propose to use the zebrafish model because it offers unique advantages for imaging studies (due to its optical transparency) and genetic dissection of signalling pathways of interest. In contrast to mammals - in which tissues need to be surgically exposed to be imaged by microscopy- zebrafish larvae can be imaged non-invasively. As a vertebrate, the zebrafish has a similar immune system to mammals/humans, while invertebrates are different and lack the immune cell types present in vertebrates. Thus, our model provides the simplest so far established animal model that can be used for studies of the cells of our interest.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We intend to perform manipulations only on embryos/larvae younger than 5 dpf (not protected under The Animals Scientific Procedures Act 1986). Adult animals (wild type or GM) will be used only for breeding purposes. The limiting factor in the number of animals used is their breeding performance. The quality of breeding activity is continuously monitored and optimised in our facility (for example through keeping a record of the size of egg clutches, avoiding repeated use of breeders in small time intervals and performing regular outcrosses). This ensures that we don't over-breed fish.
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 intend to perform manipulations only on embryos/larvae younger than 5 dpf and keep these larvae not beyond 5 dpf. At this developmental stage, the zebrafish larva is sufficiently complex for the purpose of our study yet not capable of independent feeding and complex cognitive behaviours. We will mainly work with non-pathogenic bacteria and minimise the use of zebrafish pathogens. We will perform short-term microscopy imaging (a few hours) in larvae appropriately anaesthetised, rather than long-term imaging (i.e. more than 24h).

Project 48	**Pathology of chronic inflammatory disorders**	
Key Words (max. 5 words)	Arthritis, Treatment, Alleviation, Inflammation.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our project aims at understanding the causes underlying the development of long lasting (chronic) inflammatory diseases. It has long been accepted that an aberrant response from the body's own defence system (immune system) contributes to the development of these diseases; and our goal is to analyze the nature of this abnormal immune response in order to develop drugs that can potentially re-set the immune system to normal and switch off inflammation. We are primarily, but not exclusively focusing in our research on Rheumatoid arthritis (RA) and Sjogren's syndrome (SS). RA is a chronic inflammatory disease of joints affecting 1 in 100 people. It is a severely disabling and extremely painful condition in certain patients. This condition prevents them from working, and reduces long-term cure.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	As a model of long-lasting inflammatory diseases that we focus our research on, RA is one of the most disabling joint inflammations worldwide. It affects approximately 1% of adults, reduces quality of life, increases mortality and results in large medical costs.	

project)?	National-Audit-Office figures indicate that 45% of RA patients are of working age and within 1 year of suffering the disease 30% are unemployed. RA costs the NHS/society £560 million annually (direct healthcare costs) and £4.8 billion in work- related disability. Despite the fact that several new drugs provide good results in treatment of 60-70% of RA patients, still 30-40% of patients do not benefit from these expensive drugs. This leaves a major unmet medical need and a considerable health and economic burden. By understanding the underlying causes of the abnormal behaviour of the immune system in long term inflammatory disease, we seek to: (I) Better utilize the current drugs by giving the right drug to the right patient. For example, by confirming that a specific target protein or cell is involved in causing the disease in our animal models, and finding that this target is abnormally elevated / decreased in the patient; then this patient can benefit from a category of drugs that specifically target this protein or cell. This would provide: i) better care as it would avoid delay starting a more effective drug; ii) prevent unnecessary exposure to potentially toxic drugs and iii) avoid wasting NHS money on drugs which are not going to work. (2) Develop new drugs that will target specific cells and mechanisms contributing to the development of long lasting inflammation.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mostly mice, but for specific experiments we may also use rats. We expect to use maximum 7000 animals within 5 years; 1500 rats and 5500 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?	Most of the methods we will use in this project are expected to involve mild to moderate distress for the animals. Generation and breeding of genetically modified animals will not be associated with disease manifestations observable to the naked eye and of no welfare significance. In addition, the genetic alteration will not reduce the lifespan, or prevent normal feeding and movement. Some studies involve the induction of joint inflammation in these mice which will be associated with mild to moderate joint pain, redness and swelling. Only in certain more severe protocols

	where joint pain and swelling has been induced to mimic RA are these disease manifestations expected to be severe e.g. causing difficulty for the animals to get access to food and water as normally presented. In such cases, special care will be provided e.g. by providing soft foods or easier access to food on the floor of the cage. If the condition of animals deteriorates they are killed to avoid further suffering. Drugs that induce reversible loss of sensation (Anaesthteics) will be used where possible and relevant during the studies e.g. when handling and examining animals with painful joints. Drugs that relieve pain (Analgesics) cannot be used in some cases because this would interfere with the outcome of the studies. At the end of the protocols the animals are killed and their joints, glands, and tissues of the defence (immune) system (lymph nodes, spleen, blood) are analysed under the microscope and biochemically to assess disease manifestations and confirm whether the treatment had any effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have a large group of tests that we can perform in the test tube to analyze specific immune cell interactions and functions. These experiments significantly reduce the number of conditions and molecules that require animal experimentation. However, diseases are complex and involve a great number of interactions between cells leading to the disease manifestations that can not be assembled in the test tube. We therefore use animals to look at how these cells and tissues work together, interact with each other in the whole organism to produce disease, and how they may be corrected to stop the progression of the disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A range of in vitro (‘in glass’ or ‘test tube’) testing using cells and tissues obtained from humans or animals will be set up first to define our molecular targets that may play role in inflammation and see if they can respond to our tested drugs. This strategy makes a vital contribution towards minimizing animal usage. Once animal work is deemed necessary, statistical advice will be sought in order to use the smallest group sizes possible that will provide

	statistically analyzable 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.	Although initial assessment of substances that cause inflammation can be run in tubes (in vitro), there is no in vitro model incorporating all elements of the immune system that can be used to analyse the underlying causes of inflammatory diseases. Consequently, experiments on induction, progression, and management of inflammatory disorders require in vivo (in animal) studies where the immune responses can be assessed over a period of time. Mice are the animals of choice because the reagents and genetic variations needed to analyze the mechanisms of inflammation are available in mice. In addition, there is already a great deal of prior data generated in mice that allow comparison of our results with previously published works. Finally, the entire mouse genetic map has been revealed, and future genetic interventions can be achieved. In all our experiments we are mindful of the need for refinement to avoid animal suffering without compromising the scientific integrity of the experiments. Competent personnel will perform all studies on this project licence thus minimizing animal suffering. In addition, guidelines on the limit of volumes of administration of substances and blood sampling will be strictly adhered to thus reducing the burden further. Every invasive procedure will be performed with the animal unconscious (under general anaesthesia) and painkillers (analgesics) will be given afterwards to relieve pain and ensure minimal distress and discomfort is caused to the animal. Soft pellet food and water gel pouches will be provided at the floor level to minimise suffering if the limbs of the animals are inflamed.

Project 49	**Exploiting endogenous tissue protection in inflammation**	
Key Words (max. 5 words)	Inflammation; Tissue Protection; Drug Discovery.	
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)	How does our body react to injury or infection by bacteria or viruses? How does **it** make sure that **it** responds sufficiently to kill the bacteria but not to overshoot and damage healthy tissue? in our laboratory we study the natural way inflammation (that is the response our body organises to combat injury and infection) resolves (or is switched off) such that it does not cause further damage. We propose that gaining information on the natural way this response is organised could shed vital information on 'what goes wrong' when this response continues unchecked, i.e. it becomes chronic: this is when disease is recognised. Over the years we have acquired a wealth data on the protective factors present in our blood and/or produced in our tissues (e.g. knee or muscle) when we get an injury and harm ourselves, We are now understanding how these protective factors work and can therefore control the response by our body to injury and infection. Our work indicates that in the absence of these factors, the bodys response to injury and infection is altered and inappropriate	

	leading eventually to self-harm. We intend to investigate this new biology with a dual aim: i) Understand in detail how these mechanisms operate in mammals and the processes they modulate in distinct organs and tissues (for example heart or kidney or knee) and ii) Inform how these discoveries can have an impact on making better medicines for the future.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Our laboratory is at the forefront of the preclinical and translational research into some of the protective pathways active within our body during inflammation and as such we aim to be the academic laboratory of reference for industry and biotech instructing how to harness this knowledge for innovative drug discovery approaches. As pioneers of this research line, we want to contribute to the discovery and development of novel/better therapeutics. Indeed, our research may have important implications on how to discover new medicines, as it is now appreciated that the processes we study are at the basis of several human diseases, not only arthritis but also those of the blood system and those associated with the lifestyle of our wealthy societies (e.g. diabetes, obesity).
What species and approximate numbers of animals do you expect to use over what period of time?	We will make use of rodents, over 90-95% of which will be mice. As such we plan to use — over the 5 year licence ---13,000 mice and —1,000 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?	Animals will be used in experimental protocols designed to mimic specific human diseases which have a large impact on Western societies, spanning from rheumatoid arthritis or osteoarthritis to heart disease or liver diseases. By improving the understanding of the development of these diseases and how the course of disease can be modified we aim to find new and better treatment approaches. The experimental protocols to be used are already established in our group, and have been used for many years. For example we will apply a specific inflammatory stimulus such as a chemical (as an example in the knee) and then record the development of the inflammatory response which

	involves the movement of specific cells from the blood into the knee. The majority of animals will be used in models which provoke minimal discomfort to the mice in our experience. In other models of disease, such as models of arthritis and sepsis, the suffering of the animals will be limited with the use of anaesthetics, analgesics and endpoints which predetermine the point at which animals should be euthanised to avoid unnecessary suffering. The correct number of mice will be used for each experimental group and procedure as based on statistical assays.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Much of our work is done without using living animals eg. by using of cell lines, human cells and tissues. It is not possible to provide a complete investigation of the processes of inflammation using cells alone, so animals must be used for some studies. Also, putative treatments must be tested in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The correct number of mice will be used for each experimental group and procedure as based on statistical assays Making more use of imaging (scan) means that fewer animals are needed because scans enable the progression of disease to be followed in individual animals'.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	There are no doubts to us that animal models cannot be substituted as they recapitulate the complexity of human diseases. Nonetheless, the use of animals is calibrated and thoughtful. Our intention is to develop, with this licence, experimental models of disease closer to the human situation, therefore animals will be given anaesthetics and analgesics in specific settings. This aim will not only ensure a lower welfare costs to the animals, but improve the validity of our experimental results.

Project 50	**Oral microbiology in health and disease**	
Key Words (max. 5 words)	bacteria, gum disease, inflammation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Many diseases of the mouth and intestines are not caused by a single type of bacterium but instead are a result of an upset or an imbalance in the numbers and types of our normal health associated bacteria that live in these parts of the body. This imbalance is referred to as dysbiosis. The aim of this work is to understand what factors are responsible for changing our normal health associated bacteria to disease associated bacteria. In addition the work also intends to investigate ways in which the disease associated bacteria may be changed back to the health associated type.	
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)?	Although many of the diseases of the mouth and intestine are not life threatening they are very costly in terms of treating the diseases and also in terms of the quality of life of the patients. Understanding what leads to changes in our normal bacteria and, conversely, what we could do to reverse disease associated bacteria back to the normal health associated bacteria will help in both the prevention and treatment of these diseases.	

What species and approximate numbers of animals do you expect to use over what period of time?	The Project will use up to 1900 laboratory mice that will be bred for this work over the five-year progression of these studies.
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?	For the majority of these experiments the mice will get gum disease . However, this is not a painful disease in humans and the mice are not expected to have any, or only minimal adverse effects. Other experiments will lead to the development of inflammation in the intestines which can lead to weight loss and some bleeding. The effects in some animals in these studies would be considered to be moderate severity. All animals will be killed by a humane method at the end of the experiment or if they show unexpectedly severe adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The balance of bacteria living in the mouth and intestine is due to a complex interaction between the bacteria themselves and the immune and inflammatory systems of the host organism. Understanding this interaction is one of the main of this work and therefore it is not possible to use non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All the experiments in this proposal have been carefully designed to use the absolute minimum number of animals whilst ensuring at the same time that there are sufficient to ensure meaningful results including consultation with the appropriate statistics advisors. The studies are a continuation of several years' work which has led to a clear understanding of the minimal numbers of animals required for each procedure.
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 vast majority of research that has been conducted in this area has used the mouse model of periodontal disease and inflammation of the intestine. Mice have similar bacteria to humans in the mouth and intestine and so the results are applicable to human disease. In addition there is a large number of genetically modified mice strains which will be useful in the context of understanding which components of the immune and inflammatory system are important in maintaining healthy bacteria in the mouth and intestine.

Project 51	**Development of Novel Mucosal Vaccines**	
Key Words (max. 5 words)	Vaccine, probiotic, TB, influenza, Clostridium difficile	
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
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	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 is focused on the development of highly innovative new vaccines that use bacteria as the delivery system. The long-term goals are to see these vaccines used for preventing disease in humans and in animals. Their innovation is that they can provide superior immune responses, they are heat-stable and they are safe. There exists a real and immediate need for vaccines that are heat-stable enabling simplified storage and distribution of vaccines and this is particularly so in developing countries where many people fail to receive adequate vaccination because of poor distribution and storage strategies. In developed countries non-injectable vaccine are attractive and likely to improve the quality of life. In addition, many existing vaccine carry risks associated with their use and can lead to serious side effects or poor immunogenicity requiring frequent boosters. It is with this in mind that multiple strategies for new vaccination strategies need to be addressed. In this proposal bacterial spores as well as live bacteria will be assessed for their ability to confer protection in animal models of infection. Beyond this, the aim is to see the evaluation of these vaccines in human clinical	

	trials and eventual consideration as licensed vaccines for human or animal use. All vaccines to be considered for evaluation in humans must first be assessed in animals. For this reason this project will establish the efficacy of prototype live bacterial vaccines in animal models. Our project will first design and construct prototype bacterial vaccines using existing platform technologies. Those showing the most promise with regards to their stability and capacity to express target immunogens will be evaluated for immunogenicity in animal models. We will use mice because they enable experiments to be conducted efficiently and with minimal animal suffering. In addition hamsters will be used since they are specifically recommended for evaluating vaccines to C. difficile. Animal numbers will be kept to an absolute minimum sufficient to enable subsequent publication of research results. This step is essential since it enables the most immunogenic prototypes to be evaluated further. In this project we will evaluate vaccines to *Clostridium difficile* the bacterium that causes *Clostridium difficile associated* diarrhoea (CDAD) in humans. We will also evaluate our vaccine system to other potential diseases, notably TB and Influenza. For initial experiments designed to evaluate the immunogenicity of prototype vaccines mice will be used since for vaccine evaluations mice are the only method that provides accurate data that can be considered prior to human use. Wherever possible other 'laboratory' methods will be used to reduce the use of animals in further experiments. The outcome of these experiments will be real prototype vaccine that can be utilised by industry for the benefit of public health in general.
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)?	What are the potential benefits Using C. difficile as an example, at least 50,000 people likely to derive from this per year are infected with C. difficile and >3,000 per year die from it. In the US the numbers it is estimated 1person dies from C. difficile every19 minutes. Importantly this disease is acquired in hospitals and there is no vaccine available. Clearly, a vaccine is needed quickly and an oral vaccine has been predicted to be the type of vaccine that is most

	likely to work.
What species and approximate numbers of animals do you expect to use over what period of time?	We will primarily use mice and hamsters in this work and we predict numbers of mice to be about 4,000 and hamsters about 600 over the planned 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 routine work we propose will not cause undue adverse effects. Careful monitoring will enable animals exhibiting adverse effects to be identified rapidly and these will be killed immediately by a humane procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Vaccines that are to be evaluated in humans are expected to be first assessed rigorously in animal models of infection. This is a requirement of the regulatory authorities that permit clinical trials.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have designed our experiments carefully and with sufficient numbers to reduce the possibility of failed experiments that ultimately must be repeated. Thus, careful experimental design ultimately reduces the numbers of animals 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 preferred animal model for immunological studies since they are inbred and produce the most consistent experimental data. Hamsters are required for evaluating C. difficile vaccines since they respond best to the pathogen and thus provide the most reliable data to the scientist. Only well trained and skilled staff will be used to oversee and perform animal studies. This is the single most important step in reducing animal suffering.

<table>
<tr><td>Project 52</td><td>Innate Lymphoid Cell functions in vivo</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immune responses
T cells
Innate Lymphoid Cells</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></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>
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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>Innate lymphoid cells are a recently described family of cells which appear to have important functions within the immune system. Using new mouse models, the specific mechanisms by which these cells affect immune responses will be assessed in vivo.</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 work proposed here will generate fundamental knowledge on the mechanisms by which innate lymphoid cells influence immune responses, facilitating the specific targeting of this stage of a response. This basic knowledge of how the cells affect other immune cells is essential for the development of clinical reagents to be used therapeutically, for example targeting of the molecules through which these cells regulate T cell numbers may enhance immune responses to pathogens or tumours.Conversely, where autoreactive T cells are the problem, these cells may bereduced in number or function by enhancing innate lymphoid cell effects. Mouse models where innate lymphoid cell populations are removed indicate important roles in the immune system, we need to understand the exact mechanisms to be able to manipulate this to our medical advantage.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately 7,500 mice will be required to perform the planned experiments over the five year time period.</td></tr>
<tr><td>In the context of what you</td><td>The experiments described here will reach</td></tr>
</table>

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?	moderate severity in some cases due to the surgical techniques required to precisely target specific tissues, or through the generation of bone marrow chimeras to precisely dissect the cell type responsible. Most of our models of immune response have been refined such that they are of mild severity. To specifically target certain tissues such as the mesenteric lymph node (which drains the intestine), surgery is required. This is because it is almost impossible to specifically target this tissue with conventional form of immunisation, yet to precisely dissect what is happening at this site we need an immune response to be initiated here. Animal suffering will be kept to a minimum through routine use of anaesthetics and painkillers. 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 T cell 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 objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	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 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.

<table>
<tr><td>Project 53</td><td>Antigen presentation by DCs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immune responses, infection, vaccines.</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>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>
</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 goal of this project is to understand how vaccination works to make existing vaccines more efficient and help design new vaccines against deadly diseases. In particular, we study dendritic cells (a type of phagocyte cell) that are the sentinel of the body and initiate immune responses to vaccines. Once induced, these immune responses protect us because they enable our body to respond rapidly and efficiently to pathogen encounter, or to kill tumour cells.</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 burden of most major human infectious diseases at the global scale including malaria, HIV and tuberculosis might be alleviated by the development or the improvement of immunization strategies. For example, WHO estimates that malaria infection was responsible for 627000 deaths in 2012, mostly in african children. More than 200 million people were infected. In this context, the development of efficient vaccination strategies is urgently needed.</td></tr>
</table>

	Also, cancer vaccines might boost the development of immune responses killing tumours cells. Our project might provide important information on the way to develop new vaccines against infectious diseases and tumours.
What species and approximate numbers of animals do you expect to use over what period of time?	We are using laboratory mice. The maximum of mice used during the duration of this project (5years) is estimated to 12000. Some of these mice will be used in protocols. The cells and organs of a wide number of mice will be analysed after humane killing without live experimentation.
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 wide majority of all the protocols used is mild. There is no use of chronic inflammatory disease models. In most cases, animals may experience slight fever and discomfort associated to inflammation similar to mild, self-resolving infections or vaccination in humans. A limited number of experiments involve infections or tumour growth with moderate clinical signs. Mice will be humanely killed if they display persistent clinical signs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Use of infectious model in laboratory mice bypasses the need to use primates model of infection and generate scientifically relevant knowledge. Mice models provide a unique way to test and analyse the efficiency of immune responses for 3 reasons: a) experiments are done in realistic conditions *in vivo*, b) experiments bring knowledge which is relevant for the human immune systems (because they have a lot of similarities), c) mice enable to do controlled experiments with limited numbers of individuals. Mouse experiments severely limit the development and testing of inadequate vaccines in human beings and may

	help the design of new immunotherapies.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be performed by trained and licensed experimentalists using minimal numbers of animals to generate statistically meaningful results. Multiple parameters will be analysed on each animals to reduce the number 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.	Animal pain will be limited by the use of analgesics and humane end-points will be applied when appropriate. a) Most of the biological analysis will be performed *ex vivo* after humane killing thus limiting the pain associated to experiments involving manipulations *in vivo* or surgery and the associated recovery, b) non invasive observational imaging will be used, c) non chronic disease models will be used. Highly trained scientist and license holder will perform experiments.

<table>
<tr><td>Project 54</td><td>Multigene families, immunity and virulence in malaria</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Malaria, Plasmodium, immunity</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>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>We do not know how the immune response is regulated in a malaria infection, nor the key components involved in protective immunity, immunological memory and immunopathology. The severity or virulence of malaria is determined by interactions between molecules of the malaria parasite with the host. Therefore it is important to identify the parasite molecules causing virulence. The aim of this projects is to understand the mmune response to malaria and the interactions of the host response with Plasmodium proteins in the determination of virulence using rodent models of malaria.</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 potential benefits that will derive from this project include a detailed knowledge of the protective and pathological immune responses induced by infection with the malaria parasite, and the conditions necessary to induce long-lasting immunity. This knowledge can be harnessed to</td></tr>
</table>

	develop effective vaccines. Understanding which parasite molecules are responsible for a virulent infection will allow us to design effective interventions to reduce mortality and severity of malaria.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse For the research projects of 12-15 researchers approximately 100,000 mice will be used over 5 years. 50% of these are in the breeding programme.
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 overall severity limit of this PPL is "severe". However, *the majority (60-70%) of the animals are not expected to exceed the moderate severity limit. No more than 30-40% of animals are expected to show severe signs.* Immune compromised mice or mice treated with immune substances may suffer more severe infections than normal immunologically intact mice. Hypoglycaemia and hypothermia may be observed at the peak of infection, and animals will be examined closely. If necessary the animals will be killed by a Schedule 1 method or exsanguinated under terminal anaesthesia. There may be anaemia. Blood will be monitored for signs of anaemia (haemoglobin, erythrocyte count) as part of the sampling procedure. If anaemia lasts for more than a few days the animals will be killed by a Schedule 1 method or exsanguinated under terminal anaesthesia. Mild versions of the described effects are expected between day 8 and 12 post-infection with a rapid onset and an equally rapid clearing to normal condition. A substantial proportion of the mice receiving these infections will experience symptoms such as shivering, hunched posture, pilo-erection, and become severe. The cases where the symptoms induced by the infection may reach severe (and where the mice may die) will be in those experiments where we need to establish the link between morbidity and mortality in order to gain better end point and prognostic criteria, and to

	uncover the causes of pathology or the mechanism(s) of immunopathology. If mice exhibit severe symptoms for 48hr they will be killed by a Schedule 1 method or exsanguinated under terminal anaesthesia. Monitoring during this time will be done in conjunction with staff of the Biological Services Division to ensure that the most appropriate action will be taken. *At the end of an experiment* mice will be killed by a Schedule 1 method or exsanguinated under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex immunological interaction between the malaria parasite and its host cannot be reproduced in in vitro assays. It is not possible to culture any stage of rodent parasites, nor obtain successful and reproducible invasion of liver cells or erythrocytes successfully in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will design experiments using agreed guidelines (ARRIVE) to obtain significant findings with the minimum number of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The laboratory mouse is the species of choice. This is the most refined as we have extensive knowledge of the immune system and have reagent to define immune responses. In addition there are many genetically altered mice available that allow us to ask whether defined components of the immune response are necessary for control of the parasite or for inducing pathology. Although many of the protocols described here are “severe”, our experience is that most normal immunocompetent mice do not die of malaria, but suffer a clearly observable but transient period (<48hrs) of acute clinical disease. If we are to understand the processes leading to immunopathology and the control of parasitemia of this most important infectious disease of humans, it is necessary to allow severe symptoms to manifest. We have

	previously shown that a temperature drop below 28^0C, or blood glucose level below 3mM, predict a fatal outcome. In which case we will terminate the experiment immediately. Otherwise it is impossible, at present, to predict the periods of acute/severe disease required to obtain the biological read-outs, which may become markers of clinical manifestations reflecting severe disease and/or death in humans. Therefore we cannot always intervene because if infected immunodeficient mice are culled at the first signs of severe disease there may not be time for the causative cellular and soluble mediators to reach detectable levels using the current assays. We expect that from this proposed study we will define better predictive markers of severe malarial disease, which will be immediately incorporated into our experimental work.

Project 55	**Production of Ebola virus antigen polyclonal sera**	
Key Words (max. 5 words)	Antibodies, recombinant proteins, vaccine	
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)	There is a need to expand the number of immunological reagents, available to identify Ebola virus, an important human pathogen. These reagents will be used for diagnostic tests and to monitor the output of vaccine production platforms.	
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 scientific community that is trying to develop new vaccines against Ebola virus for humans have identified the need to produce large quantities of readily available serum containing antibodies against the virus. This material can be used as a standard reagent to allow comparison between tests carried out in different laboratories and so help accelerate the development of these vaccines.	
What species and approximate numbers of animals do you expect to use over what period of time?	Rabbits – 50 Sheep – 50 Cattle - 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?	To raise antibodies against Ebola virus proteins. We do not expect to see any adverse reaction. The animals will be humanely killed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Large quantities of antibodies are required to provide standard reagents for the large number of assays that are performed to evaluate Ebola virus vaccines; this is why we have chosen to use large animals or rabbits to provide sufficient material. In vitro antibody expression systems are unlikely to produce sufficient material with reactivity against virus proteins to develop sufficiently sensitive tests.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will harvest large quantities of blood at post-mortem to keep the number of animals we use 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.	We will immunise a small number of animals using methods we have refined over a number of years.

Project 56	**Studies on the immune system and disease resistance of fish**	
Key Words (max. 5 words)	Fish, immunity, health, disease resistance.	
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
	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
	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 licence is to enable work to characterise the immune system of fish, with a view to further knowledge on defence mechanisms against infection in the most diverse group of vertebrates, and to apply• this knowledge to aid fish health in aquaculture and conservation of natural populations impacted by disease outbreaks associated with environmental change.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are three main areas that this project licence will benefit. Firstly, fundamental knowledge on how different fish species! groups defend themselves against infectious diseases. Since fish are the most numerous of the vertebrates, this will likely tell us a lot about how adaptive immunity evolved. Secondly, development of treatments to combat fish diseases in aquaculture. This is particularly important in Scotland, where farmed salmon is the largest food export and where the Scottish Government have stated a goal to increase farmed fish production by 50% by 2020. Current numbers of fish vaccinated in Scotland alone are —29M trout and 37M salmon (of —420M salmon vaccinated globally), illustrating the large numbers of animals at risk,	

	and where further protection against new and emerging diseases is needed. The information gained during the term of this project will aid vaccine design, give markers for selection and provide information on the best routes of delivery. Lastly, there is a potential benefit for conservation of native fish species, particularly in the context of understanding the impact of altered disease ranges as a consequence of environmental change, and the importance of immune gene variation at the population level.
What species and approximate numbers of animals do you expect to use over what period of time?	The main species to be used will be commercially relevant farmed fish, which in the UK is primarily Atlantic salmon and rainbow trout. Zebrafish will also be commonly used as a model species, which is used worldwide and allows comparative studies. In addition some mice and rabbits are required for antibody production (max of 20/yr and 5/yr respectively). The numbers of fish used will depend on the success at developing pilot vaccines and the need for breeding transgenic fish lines. These two factors drive the bulk of fish use. Current use in my existing project licence varies between —200 to —4,000 fish per year depending on whether field trials are being performed, and it is anticipated this will continue to be the case in the new project licence since it includes further vaccine based studies. Hence up to 4,000 fish/yr for vaccine studies are anticipated, with up to 4,000 larvae/yr for development and gene knock down studi, and <3,000 adult fish/yr for all other protocols. In the case of breeding transgenic zebrafish, eggs are produced in batches of —500, and are then grown to adults before use, leading to some thousands of fish bred over the course of the project. Due to the attrition rate of small fish fry, and the need to maintain a breeding population, far fewer fish (—10%) end up being used in experiments. For breeding transgenic fish 4,000 fish/yr are the maximum anticipated use.
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	Most of the work requires only simple procedures that will result in only mild discomfort from being handled and injected or blood sampled (whilst anaesthetised). Following venipuncture there can be expansion of skin melanophores posterior of the sampling site. This isunavoidable but does not appear to cause any discomfort. On some occasions an adjuvant will be used for immunisation, and one (Complete Freund’s adjuvant)

at the end?	may result in local effects (palpable lumps) which cause no apparent discomfort but will be monitored to ensure no ulceration occurs. When immunomodulatory molecules are studied they are typically administered in the physiological range (i.e. normal body levels) and are not expected to induce an adverse reaction in the fish. In some cases there may be sequential aspects to the experiments, where fish may undergo a treatment such as exposure to a stressor or new diet, and are subsequently tested for their immunological response. These treatments rarely have more than a mild impact on the fish. However, in some cases it is also necessary to establish the impact on disease resistance and thus fish will be exposed to live pathogens, and could die from the infection when using pathogens that induce acute pathology (eg some bacterial and viral pathogens). It is anticipated that a maximum of 20% of the adult fish may be used in such studies with acute infections, and that some 5-7% of these fish will be the controls (i.e. susceptible to infection) with potential for a severe outcome. In such cases the fish are monitored very regularly, and any showing abnormal behaviour, a proxy for being infected, are killed. In this way by the identification of early humane end points, the number of fish that actually die from disease is kept to a minimum. In addition, where possible sampling occurs before the time when mortalities are likely to occur, again reducing the chance of fish dying from infection. Lastly, in some cases manipulations to embryos will occur, especially in zebrafish using optimised methods, which could impact on their development. Any embryos? Larvae that show more than mild effects will be killed by an approved method. At the end of the studies all of the animals will be humanely killed and tissue sampled as needed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal	To study the immune response of fish it is necessary to undertake in vivo experiments, and this is particularly true for vaccination studies. In addition, very few leucocyte (white blood cell) cell lines have been developed to date for fish, so that few alternatives exist to replace the use of primary leucocyte cultures obtained by harvesting tissues

alternatives	or blood from a fish that is killed.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of fish to be used is influenced from past experiments that confirm typical variation and scale of responses seen, allowing statistical analyses, termed power analyses, to be used to optimise experimental design. I am confident we use the minimum number to achieve statistically significant results with a power of -'80%, the norm for biological studies. Nevertheless, a statistician will be consulted as required, particularly at the level of how many test groups and control groups are needed that require to be established for each individual experiment. Most of the data collected will be analysed by standard parametric and non-parametric statistical tests. One exception is experiments involving exposure to pathogens, which require a more specialised statistical analysis taking into account that each individual contributes to the "extent of exposure to risk of death" (e.g. Logrank test, Peto et al., Br. J. Cancer 35, 1977, 1-39).
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.	Most of the work will be performed with commercially relevant farmed fish species (salmon, trout), and a model species used throughout the world (zebrafish). There is already a wealth of knowledge on these species, and so in addition to being the most relevant fish to study from a UK perspective, these species give the potential to make the greatest advances. We are expert in keeping fish, with excellent aquarium facilities and trained personnel that check the fish on a daily basis, to ensure the wellbeing of our fish. Automatic alarms tell us of any problems with the water parameters, so we can get immediate help/remedies, with contingency plans in place as a precaution. A robust evaluation of the numbers of fish needed for particular experiments is carried out, and where possible in vitro assays are performed, which in some cases will be with established cell lines. As new cell lines become available during the project life they will also be evaluated for their usefulness in these studies.

Project 57	**Development of a vaccine and preclinical drug evaluation model for HIV based on humanised mice**	
Key Words (max. 5 words)	Humanised mice, HIV, vaccine, cure	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Despite extensive efforts to prevent or cure infection by the human immunodeficiency virus (HIV) novel drugs or vaccines remain elusive, largely due to the absence of a suitable small animal model mimicking disease pathogenesis. Worldwide, over 47 million people are chronically infected with HIV and require lifelong therapy. Non-human primates are currently the only in vivo model permissive to simian HIV (SHIV), a close relative to HIV. Humanised mice are xenotransplantation models based on the reconstitution of a human immune system in highly immunodeficient mice. Here, in collaboration with HIV experts we aim at improving humanised mice to enable vaccine studies against HIV. For this purpose we will 1. analyse the basic virologic and immunologic characteristics of HIV infection via different routes	

	2. determine the impact of non-redundant cytokines and growth factors on the quality and quantity of the anti-HIV immune response 3. evaluate novel drugs targeting HIV reservoirs or preventative means to validate humanised mice as a preclinical drug testing model 4. evaluate the immune response in humanised mice following injection of recombinant non-redundant cytokines 5. develop novel non-invasive imaging technologies, which may directly be translatable to humans. The use of mice for this project is mandatory since humanised mice constitute the sole small-animal model for HIV. The scope of this project including the collaborative effort 7 laboratories requires a total of 5000 animals over the duration of five years. Cohort and group sizes for experiments were calculated to obtain maximal statistical significance in all experiments while retaining the group sizes at a minimum.
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 development of a small-animal model for the evaluation of HIV drug and vaccine candidates might significantly contribute to the generation of a functional cure/vaccine for HIV, benefitting millions of infected people worldwide and limiting spread of the disease
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (including genetically altered), total number anticipated (including breeding): 6500 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?	Humanised mice might develop mild graft-versus host disease (approximately 10-20% of animals), Surgical tissue implantation may cause surgical or post-surgical complications (approximately 2%) and induction of vaginal or rectal inflammation may lead to exacerbated inflammatory responses (5%). All these conditions were classified as moderate.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The study of vaccine candidates or disease pathogenesis requires the use of in vivo model systems harbouring an immune system. Thus, utilising humanised mice is required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical calculations have been used to ascertain the minimum number of animals per group to reach statistical significance. Additionally, experiments will be conducted to ensure utilization of control groups for multiple 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.	Humanised mice and non-human primates are the sole in vivo models for HIV. Thus, we selected mice as lower species compared to non-human primates for the outlined experiments. All animals in severity grades not considered mild will be closely monitored (at least daily) to ensure minimal harm or suffering of animals. Analgesia will be provided upon signs of pain and severity limits will be strictly followed.