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Project summaries granted during 2013

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- Understanding and identification of host pathogens
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- Study of innate and adaptive immune responses in health and disease
- Engineering viral and non-viral vaccines and adjuvants
- Host defence peptides in health and disease

  Innate immunity, pathogen defence, infertility
- Environmental genetics and epigenetics in mice
- Mouse models of acute liver injury
- Pathogenesis of lung injury and organ failure

  Sepsis, inflammation, critical care, infection, organ failure
- Growth-factor-mediated effects in health and disease

  Growth-factor, regeneration, wound healing, immune response, regulation
- Neurobiology of mood and behaviour

  Attention Deficit Hyperactivity Disorder; Behaviour; Genetically-altered; Microdialysis; NK1receptor
- Development of retroviral vaccines

  HIV, Vaccine, Antibody, Immunogen, Adjuvant
- Pharmacokinetic studies to support drug discovery
- Control of Excitable Cell (Nerve Muscle) Function
- Effect of shift-work on adipose circadian rhythms

  Circadian, sleep, shift-work, desynchrony, metabolism

Harnessing local immunity for immunotherapy of lung tumours

In this project we will test whether immunising by giving a vaccine directly into the lungs, can protect against growth of a lung tumour. We will also test whether immunising simultaneously through the nose and by injection, is doubly effective.

So far, few vaccines against most tumours have been successful. This is because they need to produce immune cells not antibodies, and the cells need to be at the tumour site to be effective. In some infectious diseases, eg tuberculosis and flu, it has been shown that if immune cells are produced in the lungs by immunising via the airway, the vaccine may be more effective than when it is given by injection. This project will test whether this is the case for lung tumours as well.

So far there are no *in vitro* methods for testing immunity to the growth of a tumour, so the experiments will be carried out in mice, which we use because there are many tools for studying immune responses in this species and because the mouse immune system is similar to that of humans.

The mice will be immunised via the nose or by injection, against a molecule from a tumour and then injected with tumour cells, which go to the lungs. After an interval, the size of the tumours that develop will be measured. If immunising by the nose as well as by injection is effective, we will test whether immunising simultaneously by both routes is doubly effective. We shall then go on to test if the most effective iminunisation procedure will delay the growth of the tumour cells when the immunisation is started after the tumour cells have been injected, which is similar to the situation in patients. When we kill the mice to measure the size of the tumours we will also collect and study lymphocytes, to learn what sort of immune response is most effective against a tumour.

The experiments will involve injecting mice with vaccines or tumour cells or lightly anaesthetising them to give vaccines into the nose. Mice will be carefully monitored after tumour injection and humanely killed before the tumours become large enough to cause any distress. The smallest number of mice possible will be used that will enable us to obtain statistically reliable evidence of differences in efficacy between mice immunised in different ways. Over the duration of the project we may use up to 1,000 mice.

As lung cancer has a very poor outcome, with *<5%* of patients surviving five years after diagnosis, better treatments are urgently needed. Some vaccines are being tested in clinical trials already but these are given by injection. Data from infectious disease suggest that immunisation via the nose may be more effective against lung infections. If this is the case for lung tumours, this project might lead to an improved method of administering lung cancer vaccines and a better outlook for lung cancer patients.

Understanding and identification of host pathogens

1. The objective of this licence is to progress understanding and identification of host pathogens essential for research and delivery of' medical countermeasures, the starting point of which is the identification of candidate antigens or therapeutic targets in the microbial agents of interest. Investigation will be undertaken into the effect of targeting themes common to a number of microbial agents through vaccination or the administration of an antagonist.
2. The overarching aim is to carry out essential research investigating the development and efficacy of medical countermeasures against Biological Warfare Agents in order to develop effective treatment to be administered via the most practical route. The starting point for research and development towards a new vaccine or therapy is the identification of candidate antigens or therapeutic targets in the microbial agents of interest. Medical countermeasures to biological agents are of particular importance since they can be pathogenic in very small quantities and may take days to exert their pathogenic effects with overt clinical signs. The provision of effective medical countermeasures is a key element in bio-defence. Thus continuing the research to develop medical countermeasures, particularly those which are generic in action, is a priority.
3. The plan is to identify medical countermeasures and determine the limit of protection attainable against biological agents and toxins. Achieve non-invasive, self-administered delivery of candidate countermeasures (e.g. oral, nasal, inhalation routes) and to determine their protective efficacy in comparison with injectable routes. Determine the dosing, regimen which provides optimum protection.
4. In order to fully evaluate a candidate countermeasure, it will be necessary to consider the ultimate clinical route and means of delivery and so it will be necessary to assess both of these parameters in the whole body system using the small animal model. Where possible, *in-vitro* screening work will be carried out prior to animal work in order to minimise the number of animals used.
5. This is a generic licence potentially covering many different avenues of investigation, therefore experimental design, application of statistical modelling and power analysis will be carried out 'for all experiments to ensure the correct numbers of animals are used.
6. Animals are handled regularly and acclimatised to their surroundings. Staff are skilled in performing procedural techniques calmly and efficiently, therefore minimising any stress to the animal Once the progression of infection is understood animals will be monitored and humanely killed if it is evident that they have reached a point at which they will not recover. These signs are known as humane endpoints. Staff are experienced in monitoring the animals condition and competent in methods of euthanasia.
7. Mice are the lowest order mammalian group and have been previously well defined as the animal model for this area of investigation. Other species have been included in the event that they may demonstrate closer replication of disease in humans.
8. Multiple routes may be used in the Protocols; intra-peritoneal, intramuscular, subcutaneous, intravenous, oral, intranasal and inhalation. Minor discomfort is likely to be experienced through inoculations, administration of treatments and blood sampling, some bruising may occur. Administration of adjuvants may cause some swelling at the inoculation site. Animals will be monitored following procedures.
9, This work is of importance to UK MoD and the resultant medical countermeasures will fill capability gaps in the existing armoury of medical countermeasures to potential Biological Warfare and bioterrorist agents to protect the UK and its armed forces from threat of attack

Study of innate and adaptive immune responses in health and disease

The overall purpose of our research programme is to develop strategies for boosting natural immunity against tumours.

Our approach is to study the precise way in which experimental tumours initiate immunity, the nature of that immune response and how it becomes suppressed or inactivated by the tumour. When we have achieved sufficient understanding, we will use the information to devise ways of overcoming suppression or inactivation.

Often, when we are investigating a fundamental immunological mechanism, we will utilise non-tumour antigens thereby avoiding the use of transplantable tumours, and may undertake experiments in genetically modified mice that lack specific molecules of the immune system (for example components of the antigen processing machinery) making it possible to study the function of that molecule in more detail. Finally, when we have identified target molecules or pathways for immunotherapy, we will test their efficacy in mouse tumour models.

Because immune responses can only develop in a fully integrated system, and cannot be induced or studied at the same level of mechanistic detail in humans, our work can only be undertaken using preclinical mouse models of cancer.

Statistical calculations will be used to determine the minimum number of animals to use per experiment to obtain statistically valid results. We will use approximately 2,500 mice per year. Environmental enrichment, good husbandry and frequent monitoring will ensure high welfare standards. Few adverse effects are anticipated but, should any occur, rapid steps will be taken to ameliorate them or humanely cull affected animals. Death is not an acceptable end-point for cancer models: We will have established end-points for humane culling before pain/distress occurs, based on accepted guidelines and pilot experiments with any new models. The genetically modified mice necessary for this study do not suffer from any adverse effect due to the genetic change.

Immunotherapy offers the benefit of treating a wide range of cancers with highly specific reagents, less toxic than conventional treatments and with potential long-term protection. Identifying targets for immunotherapy and understanding the immune processes that underpin protective immunity will permit the development of new therapies and better monitoring of their efficacy.

Engineering viral and non-viral vaccines and adjuvants

This project aims to develop a versatile vaccine platform, building on recent insights into the way the body normally recognises pathogens, and using soluble polymers as scaffolds to mimic the pathogen structure. This approach should activate the body's defence pathways and encourage the production of vaccine response against chosen co-administered antigens, providing a flexible new approach to making vaccines for a broad range of diseases. To achieve this goal with the minimum number of mice, we will first test the vaccine candidates in a rigorous set of *in vitro* assays which are designed to eliminate anything that would be unsuccessful *in vivo.* Following this a number of pilot studies will be carried out to understand expected variation and refine the model in order to define the group sizes required to achieve statistical significance, thereby avoiding the use of animals unnecessarily in the subsequent studies.

Successful candidates will be defined by their ability to induce a rapid and robust memory response to the antigen against which they are being immunised. We will use *in vitro* assays to minimise the animal numbers used under this project licence, however they cannot replace the need for *in vivo* work as *in vitro* models examine too few variables, and are unable to mimic the complexity provided by a complete integrated biological system which is required to understand the immune response to, and bio-distribution of, these vaccine candidates. Mice were chosen as they are a well understood model due the huge amount of work carried out in the species and they represent a good model for human physiology.

In a typical experiment mice will be given an intramuscular, subcutaneous, intraperitoneal or intradermal administration of our test substance on two occasions, 4 weeks apart, followed by sampling of small blood volumes on 4 occasions within 2 months of the first administration; once the final sampling time point has elapsed mice will be killed painlessly by a suitable schedule I method. Welfare of the mice is of paramount importance and while toxicities are not expected, mice will be frequently monitored for signs of pain and distress and killed by a schedule I method where this is observed; all work carried out under this licence is under a 'mild' severity limit due to the benign nature of the procedures being carried out and the non-toxic nature of the substances being administered. Overall we expect to use 1500 mice over a 5 year period.

Success of this project could lead directly to the development of vaccines against anthrax and Venezuelan equine encephalitis virus, as well as providing a platform for vaccines against a range of diseases.

Host defence peptides in health and disease

The innate immune system is well conserved throughout evolution, responds very rapidly and has broad ability to detect and protect individuals from infections and cell damage. This project seeks to determine the effect that alterations in various elements of the innate immune system has on health and wellbeing. Currently we are focussing on small host defence proteins produced at the site of infection and which in the test tube have the ability to kill microorganisms. These genes have been implicated genetically in humans in both inflammatory diseases (psoriasis, Crohn's disease, cancer), infertility (affecting sperm movement). These naturally occurring antibiotics may have a very significant impact on the body's own bacteria, which have recently been shown to have a major effect on obesity.

The effect of these genes at the organ level can only be assessed in whole animals where all the different cell types can interact. Cell culture studies in the lab will be used where possible to refine and reduce the required whole animal experiments. The mouse is the species of choice as the conserved pathways to human have been extensively studies and many mutant animals to complement this work are available. We will determine the health of animals and/or cells from animals engineered to have altered expression of these innate immune genes. We will determine whether they are more susceptible to infection; have fertility defects; have an altered inflammatory response and whether the bacteria they naturally have in their gut or elsewhere is altered. In addition we will use strains of mice that are identical except for the specific gene alteration we have created to reduce the variation within an experiment, and use Power calculations to determine the fewest animal required to reach statistical significance. We will use around 7,000 mice over *5* years the vast majority of which will be for breeding. These mice will be kept initially in an infection free facility where we expect they will not appear different to normal. To determine their susceptibility to infection they will be exposed to inflammatory agents or infections and we will give them antibiotics to clear endogenous organisms and determine what species re-establish themselves. Mice may become poorly if a pathogen infection is very robust but they will be monitored throughout the protocol for signs of distress and the experiment terminated humanely should this be evident.

Wherever possible we will use surrogate endpoints by looking at blood markers of inflammation rather than allowing the animal to go to an endpoint where suffering will be evident. Understanding how the mouse version of particular human genes work in innate immunity will allow the overall understanding of this process in health and disease. Dissecting the molecular pathways of inflammation opens the way to intelligently designed novel therapies for disease.

Environmental genetics and epigenetics in mice

This project aims to assess the potential genetic and epigenetic impacts of environmental exposures, including occupational, medical and dietary exposures. Among the adverse health effects these exposures may cause, cancer is probably the most important. Rates of cancer are increasing and there is especial concern about increased rates amongst children, a phenomenon that remains poorly understood. A more positive development is that rates of cancer survival have risen significantly in recent decades, thanks to improvements in diagnosis and treatment. However, those treatments very often include chemotherapy and/or radiotherapy, both of which are potentially genotoxic. With greater numbers of cancer survivors, comes a greater need to understand the long-term consequences of exposure to radiation and anticancer drugs.

We intend to expose mice to radiation or potentially mutagenic chemicals (such as clinically-relevant doses of anticancer drugs) or dietary factors that are associated with adverse health effects in humans. Following exposure, some exposed mice may be mated with non-exposed partners, allowing us to assess transgenerational effects — an area of growing scientific interest. Following post mortem collection of tissues from exposed mice, and any non-exposed offspring, a wide-ranging evaluation of any alterations in genetic and epigenetic status, through comparison with those of control mice, will be undertaken. This will include estimates of mutation frequency and/or assessments of gene expression and/or analysis of DNA methylation and histone modification and/or studies of copy-number variation.

It is not currently possible to replicate the complex interacting biological systems (or assess changes in them) of an organism similar to humans, without using animals. However, the power and sensitivity of the techniques we use means that dramatically fewer mice are required than would have been the case using traditional methods. Furthermore, our experience of this type of *in vivo* work and expertise in statistics means we ëan design studies in which the number of animals used is minimised. We do not anticipate the total number of mice required for the projects covered by this licence, including the offspring of directly-exposed mice, exceeding 2000.

Exposures will be limited to environmentally-relevant levels considered ‘mild’ or ‘moderate’, the latter in some cases possibly due to caution regarding poorly characterised agents or exposures. We do not anticipate any behavioural or physiological side-effects resulting from these exposures. In the event of unanticipated side-effects, mice will be humanely killed and where appropriate studies will be discontinued. The laboratory mouse is commonly used as a model to evaluate the long-term effects of exposure of humans to a wide range of environmental factors. Importantly, both species share common DNA-repair and cell cycle pathways, providing ample opportunity for extrapolations from the mouse data to humans. This well-established model is currently the only species in which these experiments can meaningfully be carried out.

Our ultimate aim is to improve understanding in this area, potentially allowing more accurate assessment of, and better management of the risks and hazards associated with human exposures to factors/agents that impact on the genome or epigenome.

Mouse models of acute liver injury

1. Why is it necessary to use animals in this project; what alternatives are available; have been considered unsuitable and why?
Acute liver injury is a complex biological process that is not properly understood at present. Much of the complexity is due to the way in which the different cell types in the liver interact. An alternative to using live animals, such as cell culture, does provides a useful system for examining receptor responses and activation parameters but cannot reproduce the complex interactions of the many different cell types seen in acute liver injury.
The study of this disease in humans is limited by the inability to obtain liver tissue at frequent intervals or in reasonable quantities due to ethical constraints.
Therefore, mouse models currently provide the only in vivo models for studying the complex biology of acute liver injury.
2. What are the kinds of animals to be used, their approximate ages and numbers involved?
Only mice will be used under this application. The mice may be from inbred strains or may be genetically altered and acquired from established suppliers/breeders or bred on site.
The age at which mice will be used for experimentation will vary according to experimental protocol (e.g. bone marrow reconstitution at 6 weeks old requires 4 weeks from transplantation to use in further experimentation), but the minimum age will be 6 weeks.
The number of mice used per protocol will be kept to a minimum, power calculations will be performed to calculate minimum numbers required for statistical analysis. A maximum of 300 animals per protocol is required (excluding breeding).
3. What checks are to be made on the animals and how frequently?
Animal welfare will be monitored by Personal Licence Holders, NACWO and NVS prior to, during and after any experimentational procedures. In the protocols with a substantial severity limit a number of humane endpoints have been clearly defined and action will be taken as and when required.
4. What will happen to the animals and what adverse effects will they suffer?
The animals may be used for further breeding stock or in experimentation. Unavoidably there may be an excess of animals due to breeding patterns/gender selection/genotype transmission, any excess animals will be identified as soon as possible and culled using Schedule 1 methods.
The aim of this project is to study the pathogenesis of acute liver failure; therefore experimental animals will suffer some degree of acute liver failure.
In mice acute liver failure presents as increased drowsiness or coma. Animals will be monitored for signs of coma or reduced food and fluid intake.
The specific adverse effects are dependant upon the protocol but all animals will be closely monitored and managed. If humane endpoints are reached then animals will culled using Schedule 1 methods or those detailed in the licence.
What are the reasons for carrying out this project?
5. This project will provide better understanding of the pathogenesis of acute liver injury. The project will investigate the progression of acute liver injury using different experimental design (causing different patterns of liver injury and failure) and in different strains of inbred and genetically altered mice. This allows for the assessment of the impact of these variables on the processes involved in liver injury and its
Models of human liver disorders will facilitate investigation of the pathogenesis of liver disease that will assist in its clinical management. The potential for translational of positive interventions (including existing and novel therapeutics) in mouse models of liver disease to treatments and/or interventions could have a significant clinical impact in human liver disease.

Pathogenesis of lung injury and organ failure

This project is designed to investigate the pathogenesis of a number of important conditions, primarily focussing on sepsis and acute respiratory distress syndrome (ARDS), which cause the deaths of many people within intensive care units, reportedly up to 40,000/year in the UK alone. The general plan for this project is to utilise ‘clinically-relevant’ in vivo models of sepsis and ARDS (plus other conditions) to investigate the disease processes and the effects of treatment. A major aim of the project is to enhance translatability (to humans) by incorporating a variety of ‘co-morbidities’ (e.g. diabetes, obesity), which are very common within patients but their influence is very rarely studied.

At present, it is impossible to produce an in vitro replica of these conditions, which involve complex interactions between different organs and the immune system. We do however intend to utilise in vitro cell studies and ex vivo isolated organs to analyse some of the basic mechanisms more precisely and thoroughly.

As far as possible we will utilize animals as efficiently as is feasible, taking multiple ‘endpoint’ samples from each animal as long as this does not compromise experiments. The maximum number of animals requested is -32,000 over *5* years, although in reality the figures are likely to be closer to 10,000 over this period, 90% of which will be mice.

The mouse is genetically well characterised and numerous research tools (antibodies, reagents etc.) are already available, including genetically modified mice. In particular circumstances it may be deemed necessary to utilise rats instead of mice (e.g. where complex physiological studies are not possible in mice, or where multiple analyses could be carried out on larger tissue samples), but it is anticipated that this would involve <10% of the procedures.

We will be utilising a number of models of sepsis/ARDS, as clinically there are a wide variety of insults that cause these conditions. These models will include acid aspiration into the lungs, live bacterial challenge, burn injury and .mechanical ventilation, amongst others. For many of the models the majority of animals will be under general terminal anaesthesia, and thus subjected to minimal suffering. However, we will also be utilising more chronic injury models in recovered animals. With induction of injury animals may become unwell and show some signs of distress. In some cases development of these symptoms (e.g. reduced mobility, diarrhoea, weight loss and rapid breathing) may be necessary to carry out the objectives. However, many scientific endpoints are likely to be achieved by terminating sufficiently early that indications of substantial distress are not reached.

At the moment there is no therapy for sepsis/ARDS beyond supporting patients within intensive care until they either die or recover spontaneously. A better understanding of the underlying mechanisms is crucial to develop effective pharmacological interventions that may reduce morbidity and mortality for patients with these frequently fatal conditions.

Growth-factor-mediated effects in health and disease

The overall aim of this project is to investigate the regulatory effects of growth-factors such as insulin-like growth factor I on the immune system and how these effects can be harnessed in order to support physiological immune processes and improve immune-mediated pathologies. 3rowth-factors are generally considered to be related to development and growth and have been used for many years to treat children with growth abnormalities. However, recently it has been demonstrated that hey also strongly regulate the immune system. Members of our group have shown that growth-factors improve tissue regeneration in skin, muscle and the heart after myocardial infarct and most strikingly that growth-factor treatment can suppress the development of autoimmune conditions. We aim to further characterise this potential regulatory role of growth-factors on immune responses.

To this end, we will use established animal models for tissue damage, hyper-inflammatory and autoimmune diseases and transplantation. Animals will undergo growth-factor therapy and the beneficial effects such as improved wound healing, delayed onset and/or decreased severity of inflammatory conditions or improved graft acceptance will be assessed. In all these models, an aberrant or over-active immune response can lead to severe pathology. Due to the potent regulatory effects of growth-factors on the immune system, they have a very promising therapeutic potential in these clinical settings.

Large parts of this research project will be performed *in vitro* on isolated organs and cells. However, unfortunately it is still impossible to produce a meaningful *in vitro* replica of the complete system of molecular, cellular, physiological and behavioral interactions that happen during *in vivo* processes. This project involves such complex multi-dimensional processes and evaluation of organ function and therapeutic potential in a setting mimicking the clinical situation of human disease, is currently only possible using *in vivo* models.

Mice are a suitable model for exploratory pre-clinical investigations. They are universally used for this kind of research work and the standard protocols, methods and reagents have been optimised for this species, thus ensuring most efficient use of animals. To safeguard animal welfare, we will follow general rules of good laboratory practice and published guidelines for the work with research animals. We will also stay up- to-date on potential novel techniques in order to increase the 3Rs impact during the course of this project. We expect to use a maximum of 17.000 animals during the 5 years of this project, the majority of which will only undergo mild protocols.

As an immediate benefit, we expect this project to produce valuable information for both pre-clinical and clinical scientists. The long-term benefits of this project will be the development of new therapeutic options to improve tissue regeneration and treat hyper-inflammatory conditions. There is a major need for treatments that improve diagnosis after myocardial infarction, as well as the quality of life in patients suffering from diseases of the immune system. Understanding the molecular and immunological effects of growth-factors will allow us to extend the clinical use of this already approved and safely administered

Neurobiology of mood and behaviour

Attention Deficit Hyperactivity Disorder (ADHD) affects about *5%* of children and 3% of adults, worldwide. As well as impaired cognition, there is a high risk of suicide, criminality, substance abuse (especially alcoholism) and co-morbidity with other disorders (e.g., autism & depression). Only three drugs are licensed in the UK for treating ADHD: these include d-amphetamine and methylphenidate. There is some unease about using these drugs in children because they are both 'psychostimulants' and little is known about their long-term effects (e.g. on brain development and risk of dependence).

There is extensive evidence that the symptoms of AJJHD arise from an imbalance in the influence of neurotransmitters that govern mood, arousal and motor performance. These include monoamine transmitters (dopamine, serotonin and noradrenaline) and glutamate. The influence of all these transmitters is increased by d-amphetamine and methylphenidate. Several years ago, we discovered that genetically- modified mice ('NK1R-/-'), with functional ablation of so-called NK1 receptors, express the core diagnostic abnormalities of ADH]): namely hyperactivity, inattentiveness and impulsivity. Also, the regulation of monoamine release in their brain is disrupted in ways that are consistent with their behavioural abnormalities. Here, we shall consolidate the behavioural and neurochemical profile of the NK1 R-/- mouse and investigate whether any of these abnormalities can be prevented by established or putative drug treatments for ADHD.

Experiments to meet our specific objectives will be carried out in parallel because none is dependent on the outcome of any other. The first is to extend the neurochemical and behavioural profile of NK1R-/-, looking for factors analogous to those seen in ADHD (e.g. abnormal processing of 'novel' environmental stimuli). Secondly, we shall continue to test the effects of established drug treatments for ADHD before investigating the effects of further compounds for which there is strong scientific rationale to predict a beneficial effect in ADHD. The final objective is to explore whether disruption of functional interactions between the so-called 'brain renin-angiotensin system' and NK1R could contribute to ADHD.

Whenever possible, our experiments will follow a randomised, multi-factorial design so as to minimise the use of experimental animals. We believe we shall need a maximum of 5000 mice and 50 rats. Most of these animals will experience mild discomfort associated with drug administration and / or exposure to a novel environment. Some (about 250) will experience food-deprivation but their body weight will be monitored daily to ensure that it does not fall below 90% free-feeding weight. The remaining subjects will be used for microdialysis: the part of this procedure most likely to cause discomfort is the trepanation (a procedure commonly applied to humans) but this will be ameliorated by application of local anaesthetic.

This work can be done only in sentient animals. Most experiments will use mice because this is the species in which the genome has been mapped and a substrain has been developed that lacks the gene of interest to us. The results of this project should help us to understand the neurobiology of ADHD and might point the way to new classes of drug treatments for this condition

Development of retroviral vaccines

Retroviruses comprise a large family of enveloped RNA viruses which are natural pathogens of vertebrates, including man, who may harbour human T-cell leukemia viruses, human foamy virus and the human immunodeficiency viruses HIV-l and HIV-2.

According to the latest figures from UNAIDS (December 2010), 34 million people were living with HIV. Although antiretroviral drugs are available to treat the symptoms of HIV infection, they are unable to eliminate infection or prevent its transmission and there is a widespread belief that the best hope for controlling the spread of HIV/AIDS is through the deployment of an effective vaccination strategy. Despite nearly 25 years of intense effort, the development of such vaccines has proved elusive, although the results from a recent very large Phase ill trial in Thailand has shown limited efficacy. To date, the only form of immunity that has been shown to confer protection from infection is through neutralising antibodies (Nab), although cytotoxic T-lymphocytes undoubtedly play a role.

The Nab target of HIV- 1, and presumably other human retroviruses, is the envelope glycoprotein ("Env"). Immunisation of animals and man with recombinant forms of HIV Env results in the production of high titre antibodies, but the breadth and potency of these responses is generally narrow due to the ability of the virus to avoid and evade the human humoral response. It has not yet been fully established whether the Env epitopes of other retroviruses can induce neutralising antibodies. However, if vaccines or antibodies are to be developed against any of these pathogens, animal studies will be required.

The primary objective of this project is to develop novel vaccine immunogens and vaccination strategies against human viral and bacterial pathogens with a particular focus on the generation of specific Nabs against the envelope of HIV-l. Two research areas are pertinent to this; (1) antigen design and (2) vaccine formulation and route of delivery.

Unfortunately, no *in vitro* system exists which can effectively model the complex *in vivo* response to viral and bacterial pathogens. The immune system of the mouse will generate analogous innate and adaptive responses to antigens and can be used as a screen for their effectiveness. However, the complex structure of broadly-neutralising antibodies cannot be reproduced in mice and rabbits must be used. Structural studies will be used to model antibody/antigen interactions and only leading candidates taken into animal models, thus reducing the numbers used. I estimate that no more than 3000 mice and 200 rabbits will be employed over *5* years.

The protocols to be employed are very simple — deployment of vaccine formulation systemically or mucosally, blood sampling at intervals to measure immune parameters and bleeding-out under terminal anaesthesia. These protocols, in general, are of mild severity and cause little or no suffering.

Should an effective vaccine against HIV be developed, the benefits to mankind would be enormous. The formulation and delivery technology could also be rolled-out to develop other vaccines and biomarkers (particularly antibodies) used as diagnostic tools.

Pharmacokinetic studies to support drug discovery

This Project Licence has been designed to allow us to test the pharmacokinetic properties of our new medicines in rats and mice, as this will enable us to assess how suitable they may be for development as new treatments for human disease. All medicines, whether given orally or by injection, are to Some extent absorbed into the body, distributed through the blood system and eliminated by an excretory or metabolic process. These processes of absorption distribution metabolism and elimination (often referred to as Al)ML) will determine how much of a medicine remains in the body after dosing. Pharmacokinetics is the branch of pharmacology which characterises how medicines are handled by the body, and in particular it is concerned with how much medicine is present in the blood or other tissues over time following dosing. These properties will govern how much of an effect the medicine will have and therefore determine the size of the dose and how often it may need to be taken.

We will test our new medicines by dosing animals, for example by injection into a vein, and taking blood samples from a blood vessel from animals which arc anaesthetised or awake. These samples will be analysed using sensitive biochemical techniques to determine the concentration of the injected material over a period of time. From these data, mathematical analysis can be performed to calculate important parameters, such as half life (i.e. the time taken for the amount of a substance in the blood to drop by 50%) which are needed to judge how often to dose.

An additional benefit is that we will gain greater understanding of the doses we will need to use in other animal studies, where we are looking for the drug to have an effect on an experimental model relevant to human disease.

Although there have been advances in computer modelling in recent years, it is still not possible to model the pharmacokinetic properties of a new medicine without performing some animal experiments, as the process of distribution and metabolism is extremely complex and what might be considered minor changes in the structure of a new medicine could result in profound changes to the biological activity. The intended studies will only he carried out after extensive work in non-animal systems, such as human cell culture testing of potency or quality, to ensure that only the new medicines most likely to be successful are tested.

We plan to use rats and mice for our studies as they are well understood in terms of their biology and metabolism, and also they are likely to be the species used for later pharmacology studies where efficacy is tested. The first protocol will allow us to dose the animals, and then take blood samples from a blood vessel as described above. The second protocol will enable us to look at the effect of our dosing on simple markers of biological effect, such as a change in certain cell types in the blood — this allows us to get more information from a single set of animal studies early in the discovery process. For all of these studies we will ensure that the animals receive the highest standards of care, in line with the current best practice in the U.K., including the use of analgesics after minor surgery and the involvement of highly skilled technical staff and scientists in all aspects of the studies.

The protocols in this licence will enable us to effectively test and characterise new medicines which we are actively discovering and developing to address human ill-health and disease. There are many areas of unmet clinical need, ranging from oncology and cardiovascular disease to brain disease and respiratory disease for which we have active research programmes, and this licence will play an important enabling role For these activities.

Control of excitable cell (nerve/muscle) function

This project aims to characterise the fundamental biochemical and electrical pathways in cardiac and smooth muscle that are responsible for their normal contractile and electrical activity, to understand how nerve transmitters control the function of these muscles, and how other tissues closely associated with these muscles affect their function. Many of these fundamental aspects of muscle function remain unknown and this project aims to clarify them, as well as characterise the influence of external modulators.

These fundamental data will then be used to compare equivalent data from tissue with pathological function to elucidate the fundamental basis of organ dysfunction that in humans are often fatal and at other times lead to a severe reduction in quality of life. In particular the work will concentrate on the heart and the lower urinary tract (bladder and outflow) to provide the basis for understanding cardiac arrhythmias and failure, as well as overactive bladder syndrome.

This work is combined with equivalent studies using human tissue. However, because of the relative paucity of human tissue, animal experiments are necessary to provide a basis for undertaking targeted experiments on this valuable tissue source. Combined, the use of animal and human tissue will enormously speed up the identification of therapeutic targets to control pathologies associated with these organ dysfunctions. Although it may seem strange to work on two such different organ systems our previous research has shown that the fundamental controls over these tissues and the changes that occur with disease are similar. This has two advantages: i) to identify fundamental physiological and pathological processes that affect function of these and other muscular organs of the body (gut, uterus, blood vessels, airways, etc); ii) to reduce greatly the use of animals as studies of different organs may be made with the same animal.

The animals to be used (guinea-pigs, rats and mice) share many similarities with human tissue and so the extrapolation of these findings to human conditions will be effective and efficient.

The protocols used on the animals are painless and brief as we are concerned with rapidly euthenising the animal and them removing tissues for experiments.

Effect of shift-work on adipose circadian rhythms

Body clocks driving daily rhythms are important for synchronising physiological and behavioural activity with predictable changes in the internal and external environment such as light-dark cycles and daily meals. Body clocks are found in almost every tissue of the body. The main clock in the brain is synchronised with the light-dark cycles, while clocks in tissue such as liver and fat are mostly synchronised to meal timing.

During shift work, we desynchronise the timing of sleeping and eating from the light-dark cycle. It is thought that, this mistiming causes some of the negative health effects of shift work such as overweight and increased risk of developing diabetes. Because this mistiming of sleep and feeding may change the timing of body clocks in fat tissue, but not in the brain, it is thought that this is what is actually happening, be we don't know exactly how this occurs, and what this implies for the daily rhythms in the physiology of fat tissue.

This project explores three different pathways that may underlie the dysregulation of daily rhythms in fat tissue during shift –work:

1. Altered timing of feeding.
2. Altered expression of genes that affect both body clocks and physiology in fat tissue.
3. Altered timing of heat generation by fat tissue during sleep.

We will place mice, rats and voles on a shift work routine. During shift work, we will measure sleep brain waves, body temperature, feeding activity and consumption and compare changes in those measures with alterations of daily rhythms in fat tissue. We use wireless technology for the measurement of sleep brain waves, body temperature and behavioural activity, and this minimises the suffering of the animals during the shift work pattern.

While mice and rats are chosen because much is known about their biology, we also choose to use voles. Voles do not show clear daily rhythms in feeding and activity, and are ~~just~~ as active in the light and the dark. When we compare these animals to mice and rats, we can uniquely see the impact of daily rhythms. This approach is much more refined than suppressing circadian rhythms in mice and rats.

The circadian timing mechanism consists of many clocks and rhythms that all interact. Much of these interactions between these systems are comprehensive and for a large part unknown. Therefore we need study these effects of mistimed sleep in whole organisms, where the circadian timing mechanism is intact. Because we need to collect tissue, we cannot use humans and therefore we require these animal models.

We know that ~15% of the UK working population regularly works shift, and it is known that there shift workers are more likely to develop unhealthy weight gain and diabetes. While we know that fat tissue is a key tissue in developing these health problems, we know almost nothing about what happens to this tissue. This research aims at understanding the involved mechanism, with an emphasis on mistiming of body clocks and daily rhythms.