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Project summaries granted during 2013

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The role of 5hmC in CNS and cancer

Different cell types are able to execute their function by expressing a particular set of genes from the full complement they have. A set of mechanisms responsible for establishment and maintenance of required expression patterns can become defective due to genetic mutations, leading to cancer and neurological disorders among others. Our goal is to describe a particular aspect of gene regulation converging on chemical modification of DNA and identify potential areas for therapy.

Mouse is a critically important organism for pursuing these studies as it is the only mammal where efficient generation of genetic alterations is possible. As well, an extensive publication history allows comparative studies, when testing effect of mutations or drug candidates. Where feasible, we will use cell culture systems in our research. However frequently there are no cell culture models that accurately replicate the temporal and spatial interactions of the signalling pathways involved in development and cancer, or that can mimic the cellular organization of tissues and organs. Mice are widely used as the lowest species of mammal suitable for studying development and disease and their biology and genetics are well characterised.

We plan to use approximately 6000 mice in a period of five years. The vast majority of the animals are going to be used for breeding and obtaining the tissues for post-mortem analysis causing minimal distress. Small fraction of the animals will be used for evaluation of anti-tumour therapies and detailed annotations of molecular events of haematopoiesis, which will require tumour implantation and bone marrow transplantations. We minimise use of the animals by the use of statistical tools allowing estimating the minimal number of animals per experiment. We use most refined techniques available in the field to minimise animal suffering. In all scenarios cell culture work will precede the experiments with animals, making sure that molecular techniques are working reliably with a small amount of material, which in turn will reduce the number of animals required per experiment.

One strand of the planned experiments is directed towards evaluation of novel cancer therapies, with potential of developing novel anti-cancer medicines, which could impact lives of patients with certain blood cancer subtypes. The primary targets for the novel therapies will be Myelodysplastic Syndrome (MDS) and Acute Myelogenous Leukemia (AML), although recent data indicates applicability to wider spectrum of cancer subtypes as well. For MDS European registry documents average incidence rate of 4.1 per 100 000 per year. 3-year survival rate for the patients is rather poor (35%). AML has a similar incidence of 3.6 per 100 000 per year with a 5 year survival rate of 23.4%. Low survival rates indicate deficiencies in treatment strategies, calling for better understanding of the disease etiology leading the development of novel therapeutics.

Our second object is rare, terminally differentiated neuronal cell types. Our research will fill a gap in knowledge of gene expression regulation in cell types, which are not dividing but survive thorough lifetime of an organism.

The mesenchymal-epithelial balance in development and disease

In this project we will study the role of Wt1 and genes it interacts with genetically of physically in controlling the balance between the mesenchymal and epithelial states of cells. This is important for normal development and a range of diseases, including cancer and fibrosis.

Roughly speaking, cells can have two different states. Epithelial cells have tight connections with each other and can form strong connections and structures. They can be considered the building blocks of the body. Mesenchymal cells are more flexible and can move throughout the body. Cells can change their state from epithelial to mesenchymal and vice versa. This process is important during embryonic development as many cells have to move from one part of the embryo to another during development. However, when this process gets deregulated after birth, it can for instance help the formation of cancer metastases. A better understanding of this will help our understanding of these processes. We study this using the Wt1 gene as starting point; we have shown the last years this gene is central in controlling this balance. Wt1 was originally found as a gene whose mutation causes kidney cancer in young children, but is now known to be important in many other diseases as well. This is always linked to its role in controlling this mesenchymal-epithelial balance.

In our project we use a collection of genetically modified mice that are for instance mutated in genes involved in this balance, like Wt1, or models that are used as reporters (mice where we can easily monitor the expression or activity of genes) for different biological processes that are linked to it. We cross these animals to each other and study the effect of the mutations, where needed via the reporters. Since the mesenchymal-epithelial balance is especially important during embryonic development, most of our work can be done in embryos. We do however also use disease models that are linked to disturbance of the mesenchymal-epithelial balance to study the mutations in those situations.

Cells in the body don’t function alone by themselves, but in with each other .We are mainly interested in the way different cell types interact with each other and the role of this mesenchymal-epithelial balance in this. Most aspects we can only study in a whole animal. Our models need a certain level of technical refinement, always an important aspect of animal experiments, which at the moment can only be achieved in mice, so this is our model organism of choice. Over five years we think we will need approximately 25,000 mice. This is a lot, but more than 85% of these are born but cannot be used because they have the wrong combination of mutations. This is the result of the standard inheritance as described by Mendel, and not something we can do much but we have an NC3Rs project on-going to develop new transgenic technologies to reduce the number of super numerous mice. Moreover, our breeding schemes are designed in such a way that we maximise the percentage of useful animals.

The mice that can be used are mainly used for breeding to generate embryos for our analyses. Where adult mice are used they are monitored on a weekly basis to assure we can humanely cull them when they get sick to prevent unnecessary suffering. All our work is based on analysis of tissues from the embryos and mice. Where possible we use embryos and adult mice to generate new cell lines from for our future work where possible.

We hope this project will help us to better understand this mesenchymal-epithelial balance in development and diseases like cancer, to develop new and better therapies.

Pathogenesis of virus infections

This project will investigate the mechanisms by which viruses cause disease in their hosts and will develop and test new strategies to prevent and treat virus infections.

The viruses we will investigate include herpesviruses, influenza viruses and anelloviruses. Herpesviruses are widespread in nature. They establish lifelong infections in their hosts and can cause fatal lymphoproliferative disorders in immunocompromised hosts. Influenza virus infection is a major threat to global human health and causes thousands of deaths worldwide annually. Infections are controlled by vaccination and treatment with antivirals but new ways of combating infection and disease are needed. Anelloviruses are widespread in nature. They have not been definitively associated with disease but have been linked with liver and respiratory disorders. Further understanding is important in elucidating their contribution to disease burdens.

The role of virus and host encoded molecules in disease will be investigated by generating and characterising mutant viruses in wild type and transgenic mice. The efficacy of potential antiviral agents or vaccines will be tested by treating animals and infecting with virus.

In vivo viruses can infect many different cell types and the mechanisms of control involve the interaction of different cell populations, immune and non-immune. It is not possible to model this in vitro.

Prior to infecting animals, virus and host molecules will in studied in cell culture. To minimise animal use, we have developed protocols to obtain the maximum amount of data from each animal and will take advice from a statistician to ensure that we use the minimum number of animals required for statistically relevant results. Over the 5 year period, we will breed approximately 5000 transgenic animals and will use 12750 for infection studies.

The herpes viruses and anelloviruses that we are using are natural pathogens of mice. They do not infect other species and therefore disease can only be studied in this host. Influenza viruses are not known to naturally infect mice. However, studies over many years have shown that the mouse provides a good model for influenza virus infections. Overall, the wide range of reagents and numerous transgenic mouse lines make the mouse an appropriate species for in depth study of viral pathogenesis.

Mice will be lightly anaesthetised and infected or treated by intranasal instillation of a small volume containing virus or other material. Infections with herpes viruses are generally asymptomatic and >95% of wild mice are infected with anelloviruses. Influenza virus infection can cause severe symptoms including weight loss, respiratory distress and, in some cases, fatality. Animals infected with influenza virus will be closely monitored and any animals showing severe signs will be humanely culled. Wherever possible we will use doses of virus which do not cause severe symptoms and rely on other readouts of pathogenesis.

Understanding how viruses interact with and cause disease in their hosts will enable the design of novel strategies to prevent and/or treat virus disease. Our studies will provide information which will be taken to pharmaceutical companies for translation into treatment of animals and humans.

Evaluation of genetically engineered livestock

This project aims to develop new biotechnology applications of transgenic large animals.

The ability to transfer genes into the germline – genetic engineering to produce genetically altered animals – is both a powerful experimental tool to enhance our knowledge of biology and the basis to advance biomedical and agricultural industries. This project, building on previous and on-going animal, laboratory and computer based studies, aims to produce animals of a specific genotype for use in the following 3 areas, 1) novel animal models of human disease to enable development of new therapies, 2) combating animal infectious disease and increasing productivity by reducing animal wastage thus addressing global concerns of food security, and 3) to establish novel biotechnology applications.

For example, in agriculture, the welfare concerns and economic effects imposed by pathogen infection are huge; better husbandry and vaccination development can reduce this burden but cannot in themselves solve all issues therefore additional strategies are required. In the biomedical field, the ability to better evaluate new therapies would significantly add value to future patient treatment, as would enhancing the ability to produce both diagnostic and therapeutic antibodies.

In this project, genetically engineered animals will be evaluated with analysis of genotype and phenotype by biopsy (usually, skin or blood). Animals are closely monitored throughout growth and development. For many animals tissue samples are mainly collected after euthanasia. Some animals may undergo challenge with antibodies or antigenic substances in order to detect the effect of the transgene involved. All protocol work, involving breeding, transgenic analysis and gene activation will work to best practice and the minimum yet statistically powerful number of animals used for each study. In total over 5 years we are likely to study around 1000 animals.

Pigs are used as they are a major agricultural farm species. Sheep are used as they are a ‘model’ ruminant species of agricultural importance. Both are a recognised biomedical relevant species. Likely adverse effects will depend on the nature of the genetic change. Some transgenes are not predicted to generate a phenotype but for others it is impossible to predict all possible effects. Therefore all animals will be closely monitored throughout life by animal technicians with experience in this area of science with access to 24 hour veterinary cover and clinicians with experience with large animals.

This work will advance science through increasing knowledge, benefit people by providing a platform for reliable new disease treatments and address animal health within agriculture practice, specifically focussing on reducing the disease burden on farm animals.

Biomarkers of reproductive function in cattle

This project will seek to identify novel micro RNA (miRNA) biomarkers of reproductive function in livestock and at the same time investigate the roles of miRNAs in ovarian function in cattle. miRNAs are very significant controllers of global gene expression, regulating cell growth and differentiation. They have been identified as very useful biomarkers of normal tissue function as well as disease, however little work has been done on their potential use in monitoring ovarian function.

Failure to adequately identify fertile oestrus and/or establish a positive pregnancy diagnosis early after insemination in farmed animals are major contributors to the long-standing problem of low fertility rates in modern production animal systems, particularly in cattle. These limitations result in significantly reduced productivity in addition to being a major welfare issue through disease and premature culling.

In this study, large scale gene expression analyses will be used to identify potential miRNA biomarkers of oestrus and early pregnancy that could be used to improve reproductive efficiency of cattle and other livestock. In addition, an understanding will be gained of the molecular control of follicle and embryo development that could aid in identifying targets and developing strategies to treat infertility in animals and humans.

Follicular and embryo development are extremely complex processes under regulation by multiple cues from different body organs. Because adequate in vitro models that can satisfactorily address such complexities have not been developed, understanding the causes of loss of normal follicular function or abnormal embryo development can currently only be achieved by studying the live animals. Moreover, because of its unique physiology, the cow is an excellent model for the study of ovarian function and embryo development using relatively non-invasive techniques. However, we will also address the need for in vitro models by, in parallel with our in vivo studies, using cell cultures and computational model to complement our in vivo approached towards understanding miRNA regulation of reproductive processes.

Through the use of the most efficient statistical methods in each case we will minimise the number of animals used in our in vivo studies while maximising the amount and meaning of the data obtained. In addition, these studies will primarily involve minimally invasive ultrasound-based techniques for manipulation of ovarian function and sampling all of which are of standard use in field veterinary clinical practice and, as such, they have no significant or long-lasting adverse effects on the animals.

It is expected that the identification of novel biomarkers of reproductive function together with a better characterisation of molecular mechanisms involved in the development of fertile follicles and normal embryos will facilitate the development of strategies for improved monitoring of ovulatory activity during animal breeding programmes and IVF, as well as contribute to treatments of ovulation failure. In the 21st Century where both animal and human fertility is in decline this will be of benefit both commercially and socially.

Immunity and Mutagenesis by AID/APOBEC proteins

We are investigating the mechanisms that promote immunity to pathogens through the production of antibodies (protein molecules in the body fluids which confer immunity and are elicited by vaccination) and through intracellular proteins which prevent viruses and parasites from multiplying in cells. One of the key strategies of the body is to produce a diversity of antibodies that can recognize many different pathogens and so prevent infection. Diversity of recognition is achieved in animals and humans through controlled mutation of the DNA that codes for antibodies using the cell's own family of mutagenic catalysts (enzymes). These enzymes can also mutate the genetic material of invading viruses such as HIV and render them non-infective.

Mutation of the host's own genetic material is a rare but serious unintended consequence of these mechanisms that efficiently neutralize pathogens. It can lead to tumours of the blood (leukaemia and lymphoma) and could also be a factor in the development of cancer in other types of cells.

We use mouse models to study the function of molecules that induce beneficial mutagenesis in the immune system and to study the conditions under which the mutagenesis process results in an increased risk of cancer. While we investigate many aspects of immune function using in vitro techniques, understanding how the immune system regulates these processes as a whole and the details of tumour development can only be tested in animal models.

The mouse is a well defined model for the human immune system and allows the investigation of the genetic pathways that lead to disease. Our project will use mice of very well defined genetic backgrounds to test immune function in cases where some of the immune molecules are defective (mimicking human genetic diseases) or over active (like in autoimmunity and allergy).

In most cases mice will only be observed through their development and normal life span. Some will be exposed to procedures like vaccination where minimal or no harmful effects are expected, and in a small number (evaluated by statistical methods to be the minimum informative number) tumour development will be assessed under strict guidelines. In all cases animals will be monitored and killed by humane methods as soon as harmful signs are detected. All animals are maintained in a facility with a very high standard of husbandry to ensure that the studies are informative and only trained and skilled scientist will perform the procedures. Knowledge obtained from our research on the strategies that the immune system uses to generate diversity will be and is already being used to develop in vitro methodologies to replace the need to use in vivo models, and by using sophisticated genetic manipulations, the potential negative impact of the studies on the general health of the animals is kept to a minimum.

In addition to advancing our general knowledge on the function of the immune system and the mechanisms that promote cancer of the immune system, we are applying the knowledge obtained from the strategies used by immune cells to the creation in vitro of immune molecules (like antibodies) with the aim of developing effective biological reagents that might eventually be of therapeutic value to both humans and animals.

Evaluation of micro-particle distribution

This project will evaluate the presence of human stem cells and micro-particles in mice after injection by differing routes of administration. Studies in this project will evaluate both the distribution of the human stem cells and micro-particles harvested from the human stem cells throughout the body and how long they can be detected after injection.

The release of micro-particles by stem cells when they are administered may be a mechanism of how they act. It may be safer, cheaper and more beneficial to produce these micro-particles from stem cells and use them as a medicinal agent compared to the cells. Human stem cells or micro-particles will be injected into mice by a number of methods, into the brain (intracerebral), the blood stream (intravenous), muscle (intramuscular), or under the skin (subcutaneous). The distribution of the stem cells and micro-particles within different organs and the times that they can be found after administration will be evaluated. Data generated from this project licence will help to determine the doses of stem cells and micro-particles to be tested in future studies in animal models of disease.

Before the micro-particles are injected into mice, they will be characterised with various tests in vitro to determine if they could have beneficial effects. An initial test in animals, the formation of new blood vessels in a gel injected under the skin will be used initially to confirm these effects.

Methods have been previously developed to monitor human stem cells after injection into animals. In order to detect the micro-particles, this project will measure levels of mi-RNA sequences, biological molecules that perform multiple roles in the regulation and expression of genes that are contained in micro-particles, using quantitative PCR. The specific mi-RNA sequences that will be measured in the mice are only found in cells and micro-particles derived from human tissues. Quantitative PCR is very sensitive, allowing whole organs from mice to be evaluated which should help to reduce numbers of animals used. Once the PCR data has confirmed the presence of the micro-particles, further studies will determine the locations of the stem cells and micro-particles within the mouse organs by identifying human specific proteins. After these initial studies are completed, statistical analyses will be used to determine the lowest numbers of mice needed to perform further studies. It is anticipated that up to 740 mice will be used in this project.

Mice used for these experiments will not have any underlying illness or model of disease. The intracerebral injections will be performed via established surgical procedures followed by administration of drugs to reduce pain and saline to avoid dehydration. The remaining injection methods should cause little pain and mice will be monitored for evidence of discomfort. Injections will be performed with sterile instruments and solutions to reduce chances of infection.

Adult mice are being used in this project for their size, as they are large enough to allow consistent injections, yet small enough to permit efficient sampling of whole organs. This project also builds upon knowledge gained from previous stem cells studies performed with mice, avoiding the need of repeating animal studies in a different species.

Data from this project will be used in the development of micro-particles for therapeutic use in humans to treat a number of diseases, for example brain, heart, blood vessel and immune disorders as well as promoting healing in diabetic skin ulcers.

Characterization of microbial and murine genes required for mucosal homeostasis and pathogen resistance

The aims of this work are: 1) to discover and characterize mammalian genes linked to susceptibility to infection and chronic inflammatory diseases, and 2) to identify novel host-associated microbes that can be exploited as “probiotic-style” therapies to treat or prevent such infections or chronic inflammatory diseases. Over the past decade the genomic technologies available to characterize the microbial communities living on people and animals have matured dramatically. Such complex microbial communities, known as the microbiota’, can contain ‘--10 times more bacteria than eukaryotic (animal) host cells and code enormous genetic diversity. As a result, the microbiota and its metabolic products are major stimuli for the underlying host (animal or human) cells and play a significant role in our physiology, development and infection susceptibility. However, we have a very poor understanding of the functioning of our microbiota during growth, and during health and disease. Imbalances in the composition of the microbiota are associated with a growing list of infectious and inflammatory diseases and in some cases may be the primary driver (i.e. in inflammatory bowel diseases). We believe that such diseases can be treated with defined mixtures of microbes that restore balance and homeostasis to the microbiota-host interface. The overall research programme will employ state-of-the-art microbiological culturing techniques, genomic/proteomic analysis tools and in vitro and in vivo systems to study host-microbe interactions.

Inbred-laboratory mice can serve as a valuable human surrogate to study the basic interactions between the host and microbiota, and even serve as valuable pre-clinical models for therapy development. The microbiota of mice can be manipulated with antibiotic treatment or purposely designed to contain desired microbial cells or combinations of cells linked to health and disease states. We will employ immunological and metabolic measurements to clearly define the host and microbial states during disease and during treatments. Also, the host’s genetic make-up can be manipulated to create specific genetic changes in genes linked to human disease susceptibilities. We can now make transgenic mice by knocking out genes linked to disease susceptibility in humans, including those linked to infection susceptibility.

This novel approach will aim to confirm human variation studies and focuses experiments on the most likely genes implicated in disease susceptibility, therefore providing a systematic and rational approach to mapping disease genes that inherently reduces the number of mice.

The ultimate goals of the programme are to understand the molecular basis of disease in humans and mice, and develop novel therapies. The development of a number of genetic disease models will provide relevant models for testing novel therapies.

Mechanisms and modes of xenobiotic toxicity

Systems toxicology looks globally at the effects of chemicals in biological organisms to understand interactions and responses using high throughput assay techniques. The protocols proposed here involve the testing of various chemicals and/or genetically altered animals, using well established genomic methods, to investigate the system-based response to toxic stress.

Daily life exposes us all to chemicals. Many of these are found in food and household products, others are deliberately ingested for therapeutic reasons (drugs); collectively they are known as xenobiotics. At some level most xenobiotics pose a hazard to health, but the risk depends on the level of exposure. Many of the chemicals in common use are not fully tested; recent findings indicate that of 30,000 only 3500 are fully tested. This is of concern to the public and as a result organisations have been formed to address the issues. This, however, requires substantial testing on a large number of animals to assess the individual risk posed by each chemical.

In order to reduce the use of animals there is a need to develop, validate and refine appropriate chemical toxicity testing methods, and develop new biomarkers to better assess toxicity. The protocols proposed here are intended to identify these markers, along with improving our understanding of the mechanisms of toxicity.

Unfortunately the use of animals in work of this nature is essential. ‘Whilst many researchers are switching to in vitro cell culture-based systems, the work proposed here requires investigation of organ damage and genetic background across the whole organism. It is hoped, however, that through obtaining a thorough understanding of the biochemical features of the toxicity it may soon be in a position to switch to an animal-free system.

With this in mind work will be performed simultaneously in vitro and the two approaches will be compared. In addition, the protocols proposed here have been devised to minimise animal suffering and maxirnise the 3Rs (replacement, reduction and refinement) wherever possible. The inclusion of a preliminary dose finding study using single animals at each dose level provides valuable data that ensures only the minimum number of animals is used. Furthermore, the investigation of early-stage markers minimises both the time and extent of any suffering or discomfort. Any animal showing signs of distress will be terminated immediately. All procedures that are likely to cause pain, such as the removal of blood, will be carried out under general anaesthesia.

Mice and rats have been selected because they are widely established models of toxicity and there is a wealth of information available regarding their background genetics.

To summarise the project protocols, animals will be treated with one or more chemicals, or genetically modified to produce a similar effect. Biochemical measurements will be taken, both whilst the animal metabolises the chemical, and also posthumously via blood sampling and organ collection. The tissues will be used for genomic and biochemical analysis and final results placed on freely-accessible databases.

The benefits of this project are:
1. An improved understanding of the mechanisms of xenobiotic toxicity; thus providing valuable knowledge to help protect the health of the general public against xenobiotic toxicity.
2. The development and validation of novel biomarkers of toxicity
3. The refinement, reduction and replacement of animal testing via the sharing of data and development and validation of novel in vitro testing systems.

Models of neurodegenerative disease

Keywords: mice, ALS, SMA, disease therapy

This project uses genetically altered and normal mice to investigate why neurons die in neurodegenerative diseases such as motor neurone disease (MND) and spinal muscular atrophy (SMA), and test new therapeutic approaches for these diseases.
MND is a fatal disease exhibiting progressive loss of muscle function and respiratory failure. SMA is the leading genetic cause of mortality in infants and toddlers. No treatments are currently available to prevent, stop, or reverse these diseases.

In order to develop new therapies we need to investigate the basic mechanisms of disease and develop and characterise better models. For some disorders, specific alterations in genes are passed through families and these allow us to make models of the disease in cultured cells and in rodents. Much of what we know has come from looking at the functions of these altered genes in cultured cells and rodents.

Mice need to be used for this project for a number of reasons. Firstly, it is not possible to model the full complexities of these conditions using cultured cells. Secondly understanding how specific aspects of neuronal disturbance and death relate to how the whole animal functions can only be studied in living mice. Thirdly, it is important to study disease progression and this again requires animals such as mice over time scales not possible in culture, Finally, proper testing of new therapies requires the use of animal models since improvements in complex functions such as walking cannot be monitored in cell culture experiments. It is unethical to perform such tests directly in humans without first testing them in animal models.

In general we test new ideas in cell culture systems first, often using cell lines which do not require the use of animals. We can test many (thousands) of therapeutic approaches in cell culture, and from the promising ones further narrow them down using methods to predict which will be the most likely to enter the brain. Next we can assess the distribution of the drugs in normal or transgenic animals without symptoms, moving on to pilot studies to look at whether the drug has the expected effect in short term studies and finally for a small number of drugs which have successfully passed these stages, full therapeutic trials. A similar cascade is used for gene therapy experiments.

We work actively to minimize suffering. We have developed close links with the animal care staff at our facility and we actively involve them in decision-making. We use mice that behave in a very predictable way, allowing us to use fewer. For surgical techniques we will use appropriate anaesthesia and analgesia. For mice suffering from paralysis we monitor them closely and optimize their housing conditions to make them as comfortable as possible. We are actively developing methods to detect very early, subtle markers of disease onset and progression. We hope that these tests will ultimately replace experiments that currently use mice with a significant burden of disease.

Ultimately we expect to further understand why motor neurons die in these diseases and identity novel and useful therapies with the potential to treat patients where there is currently no effective therapy.

Control of ovine pulmonary adenocarcinoma

Ovine pulmonary adenocarcinoma (CPA) is a transmissible lung cancer of sheep and is caused by Jaagsiekte sheep retrovirus (JSRV). OPA causes considerable losses in some farms in the UK and in other sheep rearing countries. CPA is fatal and there are no treatments or vaccines to prevent or control it, nor is there a good test to identify individual sheep infected with JSRV. This project is a multi-pronged approach aimed towards developing control by treatment, vaccination, or managemental interventions. We will determine which parts of the virus are important in replication and/or pathogenesis and investigate how sheep respond to JSRV infection and the development of OPA tumours. We aim to identify the best components and protocols for JSRV vaccination and to develop better diagnostic tools for epidemiological studies and for specific control options such as test and cull, or disease accreditation programmes.

Initial experiments will be conducted *in vitro* to determine which work should proceed in vivo. To minimise the number of experimental animals required, each animal is used to obtain samples for all areas of the work e.g. for immunohistochemical analysis of cell types, for studying gene expression, for investigating the presence and load of JSRV and for optimising or validating potential diagnostic tests. In this way we can maximise the information obtained from a small number of animals.

Animals affected with OPA (10-4Opa) are donated by farmers as a source of virus and of samples for analysis. They are checked before transporting to confirm they are fit to travel and again after arrival and are treated by a vet, if necessary.

Experimental infection of sheep is the only way of testing whether a potential vaccine will be protective. It is also necessary for studies on JSRV pathogenesis and for obtaining samples of early tumour. Affected/infected animals are monitored regularly and euthanised before the disease becomes pronounced. Vaccinated or immunised animals suffer mild discomfort upon injection and blood sampling. Any ill effects of vaccination would be treated or the experiment stopped. We will adopt a staged approach in vaccine studies whereby challenge studies will only proceed where preliminary experiments show that the candidate vaccine elicits appropriate immune responses and does not generate any adverse effects.

A small number of mice, rabbits or sheep (<4 each) may be used to generate antisera for analysis of samples or as positive assay controls.
We aim to minimise stress or discomfort to experimental animals, e.g., sheep are housed in groups, rodents and rabbits have an enriched cage environment, and animals are handled only by experienced and competent handlers. Each study is carefully considered with expert statistical advice to use the minimum number of animals possible to give statistical power, i.e. useful results.

The experiments on this project will improve our understanding of CPA and may give useful insights into human lung cancers. More directly, the project will make advances towards our goal of controlling OPA. This is of benefit in terms of animal welfare as well as for the profitability and sustainability of sheep farming.

Pathogenesis of virus infections

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The viruses we will investigate include herpesviruses, influenza viruses and anelloviruses. Herpesviruses are widespread in nature. They establish lifelong infections in their hosts and can cause fatal lymphoproliferative disorders in immunocompromised hosts. Influenza virus infection is a major threat to global human health and causes thousands of deaths worldwide annually. Infections are controlled by vaccination and treatment with antivirals but new ways of combating infection and disease are needed. Anelloviruses are widespread in nature. They have not been definitively associated with disease but have been linked with liver and respiratory disorders. Further understanding is important in elucidating their contribution to disease burdens.

The role of virus and host encoded molecules in disease will be investigated by generating and characterising mutant viruses in wild type and transgenic mice. The efficacy of potential antiviral agents or vaccines will be tested by treating animals and infecting with virus.

In vivo viruses can infect many different cell types and the mechanisms of control involve the interaction of different cell populations, immune and non-immune. It is not possible to model this in vitro.

Prior to infecting animals, virus and host molecules will in studied in cell culture. To minimise animal use, we have developed protocols to obtain the maximum amount of data from each animal and will take advice from a statistician to ensure that we use the minimum number of animals required for statistically relevant results. Over the 5 year period, we will breed approximately 5000 transgenic animals and will use 12750 for infection studies.

The herpesviruses and anelloviruses that we are using are natural pathogens of mice. They do not infect other species and therefore disease can only be studied in this host. Influenza viruses are not known to naturally infect mice. However, studies over many years have shown that the mouse provides a good model for influenza virus infections. Overall, the wide range of reagents and numerous transgenic mouse lines make the mouse an appropriate species for in depth study of viral pathogenesis.

Mice will be lightly anaesthetised and infected or treated by intranasal instillation of a small volume containing virus or other material. Infections with herpesviruses are generally asymptomatic and >95% of wild mice are infected with anelloviruses. Influenza virus infection can cause severe symptoms including weight loss, respiratory distress and, in some cases, fatality. Animals infected with influenza virus will be closely monitored and any animals showing severe signs will be humanely culled. Wherever possible we will use doses of virus which do not cause severe symptoms and rely on other readouts of pathogenesis.

Understanding how viruses interact with and cause disease in their hosts will enable the design of novel strategies to prevent and/or treat virus disease. Our studies will provide information which will be taken to pharmaceutical companies for translation into treatment of animals and humans.

Mitochondrial diseases: pathophysiology and therapy

This project will tackle the issues of (a) understanding in vivo the disease mechanisms of and (b) developing therapy for mitochondrial disorders. Mitochondrial disorders affect the cellular organelles providing most of the energy required by cells; these maladies strike >1 in 5,000 children and adults and predominantly affect tissues with high energy requirements such as the nervous system, the heart, and the skeletal muscle.

Our project will expand the knowledge of the basic mechanisms of mitochondrial biochemical and execution pathways (such as programmed cell death, disposal of malfunctioning mitochondria, and the relationship between shape and function of these organelles), and on the consequences of their perturbation. This project involves the generation of mouse models bearing abnormal genes that are mutated in often fatal, invariably invalidating, human genetic disorders; it is anticipated that approximately 25000 mice will be used. Animals have to be used because the health-relevant, biological end-points we seek to understand: mitochondrial disorders are multisystemic, usually extremely severe, disorders, and their complexity cannot be reproduced by any *in vitro* system. In addition, to validate potential therapies that can eventually be translated to humans, it is essential to analyse their feasibility and safety *in vivo.*
Cell cultures will also be exploited where appropriate, such as for testing new pharmacologically active compounds.

Experimental protocols for animal work will ensure that the minimum possible number of animals is used, and that they endure the least possible suffering. The procedures applied under this licence include administration of pharmacologically active chemical compounds, adeno-associated viral vectors, and hematopoietic stem cells transplantation. The work will be carried out in a dedicated, state-of-the-art facility by highly trained technicians and scientists, all of whom are dedicated to the highest standards of animal welfare. The procedures to produce genetically new types of mouse, to breed them up into viable colonies and to experiment on them by administration of compounds/viral vectors/stem cells have been refined over many years in our lab. Tight control over breeding programmes means that we produce very few surplus animals, whilst robust experimental design enables us to generate statistically valid results from the minimum requirement of experimental stock. The scientists and technicians will work closely with Named Veterinary Surgeons to reduce adverse effects.

We will focus on a set of specific defects of OXPHOS-related nuclear genes introduced in recombinant mice that correspond to well-defined clinical conditions characterized by faulty OXPHOS in humans.

It is expected that at least 60% of animals will show no more than mild clinical signs, 20% will show moderate clinical signs, and 20% will show severe clinical signs. The latter may reflect the extreme severity of the corresponding human disease and the investigation in these animals is needed to define clinical and laboratory features, investigate the pathogenic mechanisms in vivo, evaluate pathophysiology and pathology of the target organs, search for disease biomarkers and monitor the effects of therapeutic interventions in vivo. The procedures the animals will undergo under this licence will be minimally invasive and include (i) metabolic manipulation by administration of special diets, including ketogenic and high fat diets, (ii) administration of chemical compounds, adeno-associated viral vectors and haematopoietic stem cell, and (iii) non-invasive behavioural tests.

The information obtained through this project will be presented in peer review journals and will inform the development of novel therapeutic interventions, which should in principle be translated to patients.

T Cells Subset in Immune Regulation

A variety of diseases are caused by the development of inappropriate immune responses directed against healthy body tissues or harmless substances instead of infectious invading organisms. Allergy and asthma are caused by the body making an immune response against harmless substances in the environment called antigens, for example from dust mites. This immune response is controlled by white blood cells called T cells that recognise small regions of the antigen, but ignore normal healthy tissues. When T cells recognise an antigen they start to divide rapidly, and the resulting cells change into much larger, active cells which can perform different functions, such as sending instructions to other immune cells or even killing infected cells directly. These different types of T cells are called T cell subsets. In graft-versus-host disease, the transplantation of bone marrow cells containing T cells between individuals results in the transferred T cells attacking recipient tissue antigens. In cancer, the natural surveillance process of the immune system fails to detect tumour antigens on new cancer cells. The studies we propose will help us understand how distinct inflammation-promoting T cell subsets and inflammation-blocking, regulatory T cells are induced and the mechanisms they use to mediate or prevent damage to tissues caused by immune responses. Our aim is to develop new vaccines to prevent or treat asthma, allergy, cancer, or graft-versus-host disease after bone marrow transplantation.

The development of an immune response involves many different cell types and organs within the body. For example inhaled substances are taken to respiratory lymph nodes, where a response is generated, and the resulting cells are then attracted back to the lung where can potentially cause disease. They also travel through the circulation, migrate to the spleen, or reside long term in diverse tissues. Thus, studies of immunity can often only be performed in an intact animal where all these events occur in a coordinated fashion. A large amount of knowledge about the immune system has already been gained from experiments with mice and rats, and the vast majority of this information applies to humans. The possibility of genetically manipulating mice has also made a huge contribution to the area. As well as improving knowledge of how the immune system works, we need to use mice to model human disease, so that we can test new types of treatment that might then be translated into human clinical applications. These new treatments could never be developed without animal testing. In our work we are seeking to develop vaccines that stimulate the particular subsets of T cells that are beneficial in particular disease states, and we can only test the effectiveness of a vaccine in an intact animal.

Our experiments so far have shown that by combining particular immune-stimulating molecules we can induce the type of immune response that is capable of killing tumour cells or which suppresses allergic responses in a highly effective way. Although these responses can only be induced in animals, the study of development and different functions of T cells can often be achieved without experiments on animals and we use this approach whenever possible. For example, we will study the differentiation of a new type of T cell called Th17 in cell culture experiments. However we will need to transfer these cells into mice in order to find out their role in the inflammation and airway changes that occur in asthma.

All the experiments we propose are expected to cause no distress or ill health to the animals, as we measure immunological parameters as the end-points to our experiments and there is no need to make the animals sick. For example, we induce lung inflammation in mice similar to that seen in asthma, but the animals do not experience respiratory distress as seen in an asthma attack in humans. It is always possible that animals may become sick and these will be killed immediately, since they are monitored on a daily basis. If any of the treatments are found to cause ill health they will not be continued. Therefore any suffering to the mice used in this project will be minimised. We use inhaled anaesthetic when administering substances where appropriate to avoid discomfort or the need for restraint. When possible we will replace animal experiments with alternatives. For example if our research progresses rapidly we hope to

translate our approaches into clinical trials in humans. We use the minimum numbers of mice required to achieve statistically significant, reliable and publishable results. Over the course of a 5 year period this might result in a total of around 2000 mice being used by our group. Mice are used because they are the smallest animal with an immune system comparable to humans and there are a full range of research tools available for this species. Genetically modified strains of mice may be used to address particular research questions so that we can understand how best to manipulate immune responses for clinical benefit in patients.

This research will result in: improved knowledge of how the immune system is regulated; a better understanding of what happens in chronic asthma that makes patients resistant to current therapy; and important steps towards the development of new/more effective immunotherapy and vaccines to treat allergy, asthma or cancer.

incidence of diabetes mellitus is increasing throughout the world and numbers are expected to reach 350 million by the year 2030. Drugs used to maintain glucose homeostasis have markedly reduced the incidence of mortality and extended the life expectancy in diabetics, however, numerous disease-associated complications that can arise are often resistant to treatment and cause considerable distress and can be life threatening to the patient, such as neuropathic pain and cardiovascular disease. Despite the differences in cause and prevalence, secondary complications, occur in both type 1 and 2 diabetes. It is estimated that 60-70% of diabetic patients will develop diabetic neuropathy complications with the prevalence increasing with the duration of diabetes. The burden on patients, carers and society of disorders such as diabetes and neuropathic pain is immense. New, safe and effective medicines are an important facet of how society approaches unmet medical need.

In order to treat diabetes it is important to understand the mechanisms that drive the onset of these complications. This project aims to study the mechanisms underlying major complications of diabetes (vascular dysfunction, gastrointestinal and urogenital disturbances, neuropathic pain and temperature dysregulation) using an established type 1 and type 2 rodent model of diabetes.

By understanding more about the disease process in these translatable animal models, there is the potential to develop novel therapies that can treat the disease and its complications or to identify patients who will respond best to particular therapies. Further the project will look for an overlap in mechanisms across complications and look for potential biomarker predictors of pain and responders to analgesia. Ultimately in the long term this work will be of potential direct benefit to diabetic patients since the novel therapies will treat those aspects of the disease that will assist in patients returning to a normal lifestyle

The proposed project will generate data that will be of interest to both research and clinical communities and lead to peer review publications and presentation in the lay media.
The majority of experiments carried out under this project licence will be in rats with up to 2250 animals expected to be used over 5 years.

Both induced diabetic rat models are of moderate severity with an expected transient hypoglycaemia followed by stable hyperglycaemia. The diabetic animals will be expected to develop typical symptoms of the disease such as drinking more, urinating more, weight loss and spontaneous pain due to neuropathy. Animals will be euthanased by a schedule 1 method and tissues removed for in vitro analysis at the end of the procedure

Animals have to be used in the studies on diabetes and complications such as neuropathic pain, because these are chronic complex conditions where the whole organism (i.e. intact nervous system) is required in order to measure for e.g. a painful response. Animal models of diabetes can be used for the study of diabetic complications that are seen clinically.
No in vitro systems are in existence that can replicate the whole functioning organism. Therefore, there is no feasible alternative that would entirely replace the use of a living animal that would allow the objectives to be met.

The number of animals used will be kept to a minimum. To reduce animal numbers a large amount of data (biochemical, pharmacological and behavioural) are generated from each animal thereby strengthening our understanding of the relationship between mechanisms and complications. Further, the majority of experiments will be performed in tissues taken from rats with established diabetes. Minimum sample sizes have been set using power analysis, generally using a significance level of 5%, a power of 80-90%, to detect a difference between

groups of 25%. The minimum number of animals has been refined under previous project licences in conjunction with a Biostatistics group.

The majority of experiments carried out under this project licence will be in rats as the induced diabetic rat models are widely accepted as good models of human disease. The type 1 rat model of diabetes is widely used and reflect changes that are seen in type 1 diabetic patients i.e. hyperglycaemia, polydypsia, dyslipidaemia, endothelial dysfunction, gastrointestinal disturbances and pain following innocuous stimuli (allodynia) and have predictive validity as drugs with partial efficacy in the clinic e.g. pregabalin are efficacious in this type 1 model. The type 2 model develops many complications seen in type 2 patients, including insulin resistance and sensitivity to anti-diabetic drugs metformin and glibenclamide

Both diabetic rat models are moderate severity and given they mimic many of the complications observed in the human condition some level of neuropathic pain is inevitable. However, experience from previous project licences using the type 1 model has shown us that the level of pain experienced by diabetic animals is not such as to cause any major changes in the welfare of the animals compared to the diabetic animals that do not develop neuropathic pain. Testable allodynia and hyperalgesia will be applied for the shortest time practicable and the animal has direct control over the duration of the noxious and innocuous stimulus applied, by removal of foot. To minimise welfare costs to diabetic animals the initial hypoglycaemia and reduced body temperature will be minimised by supplying 2% sucrose in drinking water for up to 2 days after diabetes induction and group housing animals.

Mechanisms of Ischemia/Reperfusion Injury and tolerance in liver transplantation

Liver transplantation is a highly successful approach to the treatment of organ failure. There are however two major problems in the field, which are: 1) the scarcity of organs; and 2) the fact that while modern immunosuppressive regimens effectively inhibit acute rejection, they are associated with substantial side effects that increase patient morbidity and mortality.
The overall objective of this project is to improve our understanding of the mechanisms by which liver transplants are rejected or accepted, and to investigate novel therapies that could enhance the viability of transplanted tissues and protect transplanted tissue from attack by the recipient's immune system. More specifically, we are focussing on understanding the mechanisms of ischemia/reperfusion injury and how this impacts of innate and adaptive immune responses. In addition, we are interested in elucidating the pathways involved in the establishment of allograft tolerance and how iron metabolism and the magnitude of ischemia-reperfusion injury influence its development.

One of the mains objectives of this project is to improve our understanding of the mechanisms by which liver grafts are damaged during the total ischemia time, period of time from the liver blood inflow cessed in the donor operation (stopping liver oxygenation) to the time that liver is re-perfused, just after the organ implantation in the recipient body (re-establishing the oxygenation). The consequences of all of liver tissue lesions developed during this period on the early graft function are named "ischemia-reperfusion injury" (I/R). I/R injury occurs upon reperfusion of vascularized tissue after an extended period of ischemia. I/R injury is inherent in every liver transplant and is the main cause of both initial poor function and primary non-function of liver allografts. The latter is responsible for 81% of re-transplantations during the first week after surgery. The shortage of organs has led centres to expand their criteria for the acceptance of marginal donors (e.g. organs from aged donors, non-heart-beating donors, and steatotic livers). This practice is however limited by the fact that marginal organs are more prone to develop I/R injury. Thus, minimizing the adverse effects of I/R injury could increase the number of both suitable transplantation grafts and of patients who successfully recover from liver transplantation. By determining the magnitude and quality of the intra-graft inflammatory microenvironment, I/R injury also impacts on the graft's overall immunogenicity.

Later on the liver transplant recipient follow-up, the long-term immunosuppression therapy, obligatory to keep the recipient´s immune system under control in order to protect the liver graft against its deleterious action, increase the incidence of malignancies, infections, and metabolic, cardiac and renal diseases, contributing for the higher morbidity and mortality of transplant liver recipients when compared with the general population. As a consequence, demonstration that not all human transplant recipients require perpetual immunosuppression to maintain their graft mainly derives from the subset of patients who discontinue conventional immunosuppression through non-compliance, out of medical necessity, or within drug withdrawal trials, and yet sustain normal graft function. These patients are considered as "operationally" tolerant. In kidney transplantation this fraction is estimated at <5%. For liver transplantation, in contrast, recent studies indicate that it can reach 15 to 40% or more depending on recipient age and how remote from transplant. The realization that in liver transplantation tolerance is more prevalent than previously estimated is consistent with the notion that the liver is an immunoprivileged organ, and has resulted in tolerance being recognized as a tangible clinical opportunity. There is considerable pressure therefore to delineate the mechanisms responsible for the development of liver allograft tolerance in humans. In a recently conducted clinical trial of immunosuppression withdrawal in liver transplantation it has been demonstrated that transplantation tolerance is associated with the differential expression of genes involved in the regulation of iron metabolism (e.g. *HAMP*, *TFRC*), as well as with differences in serum ferritin and hepcidin levels. These results suggest, for the first time, that the iron/hepcidin axis might be involved in the regulation of inflammatory and alloimmune responses. The interactions between

iron metabolism and innate and adaptive intra-hepatic immune responses, however, have not been adequately investigated.

The purpose of this project is to understand how modulation of iron metabolism and/or the complement cascade influences the development of I/R injury in liver transplant recipients and the subsequent development of allograft tolerance, and to investigate whether this can be modified by therapeutic strategies. The specific aims are as follows:

- To determine the contribution of specific complement components and/or complement inhibitors to the magnitude of I/R liver injury and how iron metabolism influences and modulates I/R liver injury.
- To determine the contribution of specific complement components and/or complement inhibitors to the capacity of the liver to regenerate.
- To determine if the magnitude of I/R injury influences the development of allograft tolerance in liver transplantation, and whether this can be modified through modulation of the complement cascade.
- To establish if iron metabolism is involved in intra-hepatic inflammatory responses.
- To investigate how iron metabolism influences the function of allo-reactive T cells.

To cope all of these aims described above, we will use the following previous established experimental anima models:

- Breeding and maintenance of genetically altered animals
- Rat liver transplantation
- Skin transplantation in mice and rats
- Partial hepatectomy in mice
- Ischemia-reperfusion injury in mice
- Concanavalin-A Induced hepatitis in mice
- CCL4 induced liver injury in mice
- Chronic Auto-Immune hepatitis in mice
- Induction of Graft versus Host Disease.

Our project will attempt to find solutions for these two major problems of clinical liver transplantation described above. We will investigate whether we can reduce the intensity of the I/R injury. The two linked solutions we are exploring in the current project are to increase graft viability by inhibiting ischemia-reperfusion injury and to develop means to induce immunological tolerance to the liver allograft. Therefore, the findings from this project will be important in that they may allow a liver allograft to last longer, which could impact on the clinical problem of shortage of organs. Understanding how iron metabolism regulates innate and adaptive immune responses will provide information that could be used in the clinic to facilitate the spontaneous development of tolerance following liver transplantation. Finally, demonstration of whether ischemia-reperfusion injury influences the development of transplantation tolerance will provide relevant information on how innate immunity impacts on tolerogenic adaptive immune responses and could also have clinical implications by pointing out novel strategies to facilitate the establishment of tolerance at early time points after transplantation. If our investigations are successful, we might be able to define novel therapies to allow a transplanted liver to last longer and to reduce the health problems associated with the side effects of current immunosuppressive medications.

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Regulation of stem cell function by PGF Signalling
Stem and progenitor cells have great potential in tissue regeneration and repair. However, to maximise their use in these processes, there is a need to understand the factors and mechanism that maintain and regulate stem/ progenitor cell proliferation, differentiation and commitment. Identifying these factors will allow us to tackle cell loss or cell malfunctioning due to ageing, injury or inherited degenerative diseases.

Our goal is to understand the role of one particular family of cell-to-cell communication genes, termed Fibroblast Growth Factors (FGFs) in the above processes. Led by a set of preliminary data, we want to particularly understand the role of FGFs in the biology of brain and muscle/tendon stem/ progenitor cells. To achieve this we will analyse transgenic mice in which the functionality of particular FGFs have been selectively modulated in stem/progenitor cells of these tissues, in vivo. An in vivo approach is critical because the behaviour of stem/progenitor cells is heavily influenced by factors present in their surrounding environment or niche, which are difficult to model wholly in vitro.

Typically, our experiments will involve the activation of traceable marker genes in stem/ progenitor cells at specific time points during embryonic development or in adult tissues, followed by an analysis of their progeny. e.g. The type and rate at which daughter cell are generated and their anatomical position and relationship with their neighbouring cells. By comparing the fate of such cells in mice in which FGF function as been additionally compromised (or elevated), we will be able to draw conclusions about the role of FGFs in stem/ progenitor cell biology.

Most of the animals used (16,000) are for the purpose of generating the appropriate sets of compound mutant mice needed (double and triple transgenics; in protocols 19b1 and 2). A subset (4050) will then receive compounds that switch genes on/off (19b3) or will be used in experiments that alter mouse physiology (19b3), or allow us to measure changes in physiology (19b3,4) – all within the moderate level of severity. During this work, we will take all possible measures to promote the 3R principles, by reducing animal usage through better forward planning of experiments supported by proper statistical analysis; regular review of breeding stocks; sharing tissues with colleagues and other researches; seeking ways to develop in vitro models; and minimising animal discomfort.

We anticipate that our findings will impact three important areas of human medicine: (i) Apert syndrome – a congenital disease that arises from malfunctioning of FGF signalling pathway in multiple tissues, including the brain; (ii) Muscle and tendon-related diseases arising from ageing or injury; and (iii) Neurodegenerative diseases and obesity, which, respectively, arise from lack of repair and malfunctioning of brain circuitry. Use of mice as an experimental model is justified by the remarkable anatomical and genetic similarities that exist between these and humans. Hence, our findings are most likely to be useful for strategies aimed at preventing or alleviating human disease.

Generation of anti-hapten antibodies

Establishing if life ever existed on Mars is one of the outstanding scientific questions of our time. To address this important goal, the European Space Agency (ESA), in cooperation with NASA, has established the ExoMars Programme to investigate the Martian environment and to demonstrate new technologies paving the way for a future Mars sample return mission in the 2020's. The company has been asked to collaborate with the Life Marker Chip (LMC) Project within the ExoMars consortium (EMC) in the generation of monoclonal antibodies to be used as part of the testing for life on Mars. Work by other collaborators has not been successful and the company was approached as world leader in the field of monoclonal antibody generation.

The purpose of this project is to generate antibodies to simple hapten molecules that may represent some of the earliest molecular relics of the most primitive life. If successful it will provide tools for the detection of these molecules to aid in the understanding of the development of life. These will then be applied in the detection of possible extraterrestrial life on Mars or detection of primitive life in extreme environments on Earth. In either scenario, it would generate useful information about the earliest stages for development of life.

The company is using its expertise in monoclonal antibody generation to help the LMC Project within the European ExoMars Consortium. We will develop the technology to generate monoclonal antibodies to weakly immunogenic haptens using two of the haptens from their panel. The methodology will then be transferred to the LMC to continue the antibody generation work with their other external collaborators. This will allow them to successfully generate antibodies to the complete hapten panel that, as stated previously, could indicate the presence of molecules that are associated with primitive life or are precursors to life on the Martian surface.

In order to generate the antisera and ultimately monoclonal antibodies to these compounds, they will be conjugated to carrier proteins such as BSA, transferrin or KLH for immunisation using conventional coupling chemistry. Immunisation and screening will be performed according to conventional approved procedures.

As the aim of this project work is for the company to transfer a method to our collaborators that is suitable for making monoclonal antibodies to difficult hapten molecules, whilst using methods that are available in the public domain, it is not possible to do this without using animals. Based on our experience of generating antibodies to targets that are poorly immunogenic, a specific hyper-immune mouse strain (called SJL) will be used in combination with a rapid immunisation protocol (called RIMMS). This will maximise the chances of generating an immune response to these difficult haptens and increase the probability of isolating useful antibodies. The main advantages of these two options are listed below:

- The hyperimmune status of the mice enhances any antibody response and makes weakly immunogenic targets more likely to stimulate the immune system
- The direct immunisation of the antigen adjacent to the lymph nodes improves the antigen delivery and presentation to the immune system

To immunise the animals they are anaesthetised, and antigen injected subcutaneously at sites adjacent to the major draining lymph nodes. This is repeated several times over a 2-3 week period in order to maximise the stimulation of the immune system. Immune cells are then harvested for hybridoma generation for monoclonal antibodies.

This approach to quality for immunisation ensures that the minimum number of animal required for the project work will be used. It is anticipated that completion of this piece of work should require less than 100 mice.

This work may provide fundamental evidence of whether these molecules are present on the Martian surface. The outcome will be of great value to the scientific community in understanding how Mars has developed. The antibodies can also be used to show if these simple molecular compounds are present in extreme environments on earth, to again aid the understanding of development of life on Earth.

Germ Line, Stem Cells and Epigenesis

Germ cells have the unique property of transmission of genetic and epigenetic information, and this provides an enduring link between all generations. We seek to understand how precisely germ cells are formed during early development, and the role of key genes in the process, which result in germ cells being set aside from the rest of the cells that will develop into different tissues and organs in adults.

During development, germ cells must undergo specific changes that result in them acquiring the property of totipotency, which is the ability to give rise to a whole new organism after fertilisation, and therefore of all generations. For this reason germ cells are considered to be 'immortal' unlike somatic tissues, which perish with each generation. Some of the critical events start as soon as germ cell are formed. Amongst the major early events is the process of comprehensive erasure and resetting of epigenetic information that will allow germ cells to develop with the unique ability to give rise to a new organism after fertilisation. The epigenetic information itself takes the form of specific modifications of the DNA or of the proteins bound to DNA but without affecting the DNA sequence or genes themselves. Epigenetic changes regulate gene expression, turning them on or off at the appropriate time. In germ cells, all the previously acquired epigenetic information needs to be comprehensively erased, which is crucial in order to restore totipotency.

We have acquired knowledge of the key genes that are involved in specifying germ cells, as well as of the factors that are involved in the erasure of epigenetic information. Further research is aimed at elucidating the precise mechanisms of how these factors work individually and together during the formation of germ cells.
Information gained from this project may be widely applicable in regenerative medicine, manipulation of cell fates, and for alleviating age-related human diseases. For example, mechanisms of erasure of epigenetic modifications might find a role in rejuvenation of cells and tissues affected by ageing. We are also developing methods whereby we can generate germ cells from pluripotent stem cells. With further work, we might be able to achieve this with the use of human pluripotent stem cells, which could pave the way to a comprehensive studies on human germ cells for the first time, and lead to advances in reproductive medicine and on revealing the basis for the formation of germ cell tumours.

There are also reports suggesting that not only genetic mutations, but environmental and dietary factors that adults are exposed to, can adversely affect not only the germ cells in adults, but this can affect development of their progeny, as well as subsequent generations. It is thought that such environmental factors induce adverse epigenetic changes in germ cells, which can be heritable and transmitted through the germ line. Some human diseases are suggested to occur through such inheritance of inappropriate epigenetic information. While these observations need to be further explored, it is important to understand the mechanisms of such transgenerational inheritance through the germ line since they may have significant consequences for human health. Deep mechanistic understanding of the germ cell lineage is required to explore this possibility.

Much of this work can be done *in vitro*, but some aspects require mammalian model. We will use mice, as they are a well-studied model, amenable to generic alteration and sufficiently similar to other mammals to be an informative model of human development.