HOME OFFICE LICENSING

Since the last meeting, the ERP Certificate Holder's Advisory Group has recommended 6 applications for amendments to existing project licences and 2 applications for continuation project licences. The Committee is asked to receive and note these applications, which were:

Application

1. Project Title: **Investigation of the avian visuomotor system**

An application for a new Project Licence. The application has been granted by the Home Office, and the project abstract is reproduced below for further information.

2. Project Title: **The genetic control of cancer**

An application to continue work from an existing Project Licence. The application is currently with the Home Office, and the project abstract is reproduced below for further information.

Amendments:

1. Project Title: Cell signalling, survival and differentiation in neurodegeneration and neoplasia

An application to amend an existing Project Licence. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

2. Project Title: **Development and Plasticity of the Visual Cortex**

An application to amend an existing Project Licence. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

3. Project Title: Role of Innate Immunity in disease of the Central Nervous System

An application to amend an existing Project licence. The application is currently with the Home Office, and the lay summary is reproduced below for further information.

4. Project Title: Arthritis: Pathological mechanisms and Therapeutic intervention

An application to amend an existing Project licence. The application is currently with the Home Office, and the lay summary is reproduced below for further information.

5. Project Title: Immune responses during persistent virus infection

An application to amend an existing Project Licence. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

6. Project Title: Aquatic contaminants and nutrient sources in aquatic birds

An application to extend an existing Project Licence. The application is currently with the Home Office. The applicant drew up the licence originally for three years as a visitor from Canada and has achieved the four original objectives. Two further objectives were added to the licence as an amendment in 2008 in anticipation of extending her stay, and as she is now able to do this, the extension will be used to address additional objectives fully.

BSO – 8 March 2010

Lay Summaries and Abstracts from Applications Processed by CHAG

Please note – These are for information only – no assessment by the Committee is needed

Application 1 – Investigation of the avian visuomotor system

Abstract: The principal goal of this research program is to improve our understanding of the functional organisation of the brain pathways controlling visuomotor behaviour (i.e. accommodation, eye movements, regulation of retinal activity) in vertebrates and to use this information to determine the role that the central nervous system may play in influencing eye growth and the development of refractive error, such as myopia.

There are no in vitro models for myopia induction, investigations of intact visuomotor brain pathways, or assessment of motor behaviour or visual function, including eye phenotyping, necessitating the use of a living animal model. The organisation and layout of these pathways as well as their complex interconnections with the functioning eye prevent the development of a single brain slice (i.e. in vitro) preparation. Therefore, the complete, interacting pathways and their physiological function and/or impact on eye development need to be determined and studied in a live adult or developing animal.

Because the visuomotor system has largely been conserved in the course of evolution, a number of animal models (i.e. much lower sentience than primates) can be used, and the results are likely to have relevance to the relevant human condition. Avian models have long been established for the study of both eye development (chicks) and brainstem pathways controlling and mediating visuomotor behaviour (pigeons and chickens). In fact, birds are the least sentient vertebrates that rely on vision as their primary sense, in marked contrast to rodents. Specifically, chicks have become a widely used, validated model for the study of visually induced myopia.

The design of the proposed anatomical and microstimulation experiments involves both experimental and control groups. In all of the proposed experiments, the number of animals requested reflects the need for an adequate sample size to allow meaningful statistical comparisons between these groups, where required. The completely crossed visual system of birds makes it possible to make *within-animal* comparisons, greatly enhancing the statistical power of the experimental design and thus significantly reducing the number of animals required for each group.

Epidemiological studies in humans strongly suggest that overuse of accommodation and/or accommodative dysfunction plays a role in abnormal eye growth, leading to the development of myopia. Moreover, the centrifugal visual system, found in all vertebrates, can modulate retinal activity, which in turn has also been shown to influence eye growth. The proposed research will provide (1) a better knowledge of the brain pathways (especially their respective inputs from other brain centres) involved in these visuomotor systems, and (2) direct information about the influence of the activity of these pathways on eye growth. An understanding of what goes wrong in the brain or eye may lead to a future cure for high myopia by halting excessive enlargement of the eye during childhood. The research may also shed light on the underlying causes of anisometropia (i.e. asymmetric refractive development of the two eyes), which (when severe) can produce amblyopia.

The recent demonstration of sensory feedback from the extraocular muscles has extremely important implications for surgery carried out on humans to correct a variety of oculomotor pathologies such as squint, nystagmus, and thyroid eye disease. A better understanding of the role of the fine structure and visuomotor

control of eye muscles, as well as the response to any sensory input from the muscles, will facilitate future refinements in extraocular surgical interventions in humans, especially children.

Application 2 – The genetic control of cancer

Abstract: This project aims to enhance our understanding of the basic mechanisms underlying epithelial tumourigenesis and to utilise this knowledge to develop and test new therapies. This will be achieved by generating mice models bearing precise genetic mutations implicated in human cancer. Currently there is an urgent need to develop novel strategies to a range of human tumours, a substantial proportion of which remain refractory to current therapies. A better understanding of the genetic basis of disease, and of the mechanisms which drive tumourigenesis, will help identify new therapeutic targets. The generation of ever more precise models of human cancer will also speed the translational pipeline, as they will permit more rapid and accurate readout of *in vivo* drug activity. These aims will be achieved by building and studying novel models that recapitulate the multiple genetic lesions occurring in human disease, and by subsequently treating these models with novel therapeutics. These studies cannot be replaced by *in vitro* systems, as a critical feature of the models is that they are autochthonous- ie that the tumours develop in the correct cell and tissue type, and that they do so in the presence of a functional immune system. We can however, recreate some aspects of these studies in vitro, for example by isolating and growing cancer stem cells. We will pursue these studies where appropriate, but they will augment rather than replace the in vivo analyses. Minimum group sizes will be identified by power analyses. Mouse numbers will be minimised because the genetic tools we will use will be defined and precise recapitulations of the human situation. We will also use protocols to minimise animal use where possible, for example through the use of colonoscopy and MRI imaging. These studies will be performed in the mouse because this is currently the only mammalian system where such extensive genetic manipulation is possible. These experiments utilise mice mutant in genes that control neoplastic predisposition. We are also investigating the response to various drugs, including novel chemotherapeutics. As such, the various drug/strain combinations are predicted to have altered responses to drug challenge and an altered predisposition to neoplasia. The mice may also have altered developmental programmes as the primary function of many tumour-related genes is to control the normal development. This work is aimed at developing strategies that will prevent or cure human cancers. There are two important potential outcomes for human health. The first is the identification and in vivo validation of new therapeutic targets; the second is the testing and validation of new therapeutics. Both of these outcomes have the potential to significantly impact on human health.

Amendment 1 – Cell signalling, survival and differentiation in neurodegeneration and neoplasia

1. Lay summary: What the changes are:

Clarification of clinical signs of paralysis that require immediate action was added to Section 19b

2. Why the changes are needed:

To avoid any discrepancy in interpretation of the severity status by different people involved in animal maintenance.

3. Species and number to be used

Mice already included on the licence, no changes introduced.

4. Effects on the animals:

Will help avoiding situations when deterioration of animal health might exceed permitted moderate level.

5. Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

Not applicable

b) Potential benefits:

Will help avoiding situations when deterioration of animal health might exceed permitted moderate level.

c) How the benefits outweigh the costs:

It would be less chance of unnecessary suffering.

Amendment 2 – Development and Plasticity of the Visual Cortex

Lay summary:

1. What the changes are:

1) A change to the list of genetically altered animals in Procedure 19b.8 (v): the two specific knock-out mouse strains have been replaced with a broader reference to "Mice with genetic alterations of interest to the scientific investigations described in this project licence"

2) Addition of optional implantation, under Procedure 19b.8 (v), of a micro-electrode for electrophysiological recordings

2. Why the changes are needed:

1) Other mouse strains with mutations that affect glutamate receptor mediated signalling and its role in visual cortical plasticity have become available. Their use will aid in achieving the scientific objectives of this project licence.

2) The ability to record visually evoked potentials in parallel with optical recordings of cortical activity will cross-validate the results from longitudinal assessments.

3. Species and number to be used

1) and 2) No change in the species (mouse) or number of animals to be used.

4. Effects on the animals:

1) and 2) No change in effects on animals (see **5a** below).

5. Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

1) There are no additional costs to the animals.

2) There are no additional costs to the animals. Animals have already undergone surgical preparation under general anaesthesia incl. craniotomy for implantation of an osmotic minipump (which similarly requires insertion of a needle into the exposed cortex), therefore there will be no additional adverse effects.

b) Potential benefits:

1) The use of additional genetically altered mouse strains could shed more light on the role of glutamate receptor mediated signalling in visual cortical plasticity.

2) Longitudinal assessment using two different recording techniques will strengthen the validity of any results obtained.

6. How the benefits outweigh the costs:

The improved likelihood of achieving the objectives of the project outweighs the unchanged costs to the animals.

Amendment 3 – Role of Innate Immunity in disease of the Central Nervous System

Lay summary:

1. What the changes are:

The major change is the addition of a new objective and an extra protocol to allow us to carry out the objective.

Secondly we have removed the line "Animals will be bled no more than once a week for the duration of the experiment" from all protocols.

Thirdly we have extended the times required for some of the scanning.

Lastly we have changed the deputy since the incumbent is no longer employed within Cardiff University.

2. Why the changes are needed:

The new objective is built around the need to commission the All Wales PET scanner, which is due to be operational, by the end of April.

We have removed the line (detailed above) from our protocols since this was included in error and only noted recently when we wished to begin testing some of our therapeutics using procedures detailed in this licence. We need to monitor carefully over the initial period (hours) after administration how long our therapeutics remains in circulation. This gives us important data on dosage required and frequency of administration. Stating that we shall only take blood samples once a week obviously precludes us from gaining this important information about our potential new drugs.

Due to the need to scan some animals for longer periods than was anticipated then the licence was written we have amended the times cover the required time interval.

3. Species and number to be used

24 additional adult mice and 24 additional adult rats in total to be used in the new 4th protocol over the remaining three and a half years of the licence. While we require only 18 of each to commission the PET scanner, we have include 6 extra animals of each species in order to cover any unforeseen circumstances which might require reputation of a particular set of scans.

4. Effects on the animals:

For the new protocol the animals will be under general anaesthesia for the duration of the procedure, at the end of which they will be subject to terminal anaesthesia using an overdose of anaesthetic. During the period under anaesthesia they will be administered radiotracers and scanned via PET. Heartbeat and respiration will be monitored during this period.

Extra sampling of blood as requested by removal of the restrictive line referring to sampling once per week should have little effect on the animals. Total blood volume withdrawal within any 24h period will still remain at no more than 10% and no more than 15% within a 28day period. We simply need a number of small volume samples at several time points.

A small number of animals will be scanned for a longer period (up to 90mins), remaining under anaesthesia for the duration. The great majority will still be scanned under anaesthesia for 15-30mins.

Brief summary of the cost-benefit ratio of the proposal:

5. Costs to the animals:

18 rats and 18 mice (with leeway to use 6 more of each) will be subject to terminal anaesthesia and utilised as above, at no stage will they regain consciousness during this procedure. Hence there will be no adverse effects upon conscious animals.

Increasing the number of blood samplings taken from each animal during a procedure may incur the animal some discomfort, this will be minimised through the use of local anaesthesia and good practice e.g. applying pressure to "tail nicks" after sampling in order to rapidly halt bleeding.

Increasing the length of scanning for some animals should have little effect on their health, they will remain under general anaesthesia during this period and their heart rate and respiration will be continually monitored.

6. Potential benefits:

This work will allow us to fully commission and validate the new PET scanner and open the way for its usage by many local and other researchers working in a number of different fields relating to human health, e.g. cancer biology, cardiovascular research, and Alzheimer's.

Being able to take a number of small volume blood samples from animals where we are testing new therapeutic reagents will allow us to properly evaluate and test the efficacy of our reagents. Such stringent testing is required if we are to progress any potential therapeutics further.

Increasing the length of scanning for some animals is a requirement to gain good data from some sorts of scan that are to be used. The data gained with these longer scans, in conjunction with that from other scanning modalities strengthens greatly the overall picture gained.

7. How the benefits outweigh the costs:

Having a properly commissioned PET scanner available will benefit research across a number of fields as touched upon above. Several groups within the Cardiff University currently have plans to use the facility. A fully commissioned PET will allow researchers to monitor disease progress and assess the efficacy of new therapeutics within living animals over extended periods of time since the same animal can be re-scanned days or weeks later. The data gained through the unavailability and use of this new facility will enhance our understanding of the basic mechanisms that drive progressive diseases such as Alzheimer's, heart disease and cancer.

This technology will have knock on benefits for animal welfare since being able to scan the same animal repeatedly will reduce the need for animals at each time point hence lowering the total number of animals required to complete a study.

Blood sampling is crucial to both the monitoring of radiotracers and the proper testing of new therapeutics. The total volume taken from each animal will not change.

Increasing the scanning time for a small number of anaesthetised animals will have few if any adverse effects but greatly increase the power of the overall data obtained.

Amendment 4 – Arthritis: Pathological Mechanisms

Lay summary:

1. What the changes are:

(i) Additional protocol 19b 6 (experimental osteoporosis) - to allow us to identify molecules that precipitate loss of bone mass, obtain a greater appreciation of the mechanisms by which these molecules regulate bone turnover and to utilise this information to develop alternative novel targeted therapeutics for osteoporosis.

(ii) Permission to use novel imaging techniques to assess arthritis – to facilitate a reduction in animal usage by performing longitudinal studies.

(iii) Change from mouse to rodent (19b protocol 1, 2 and 5) – to allow flexibility in using mice or rats for the protocols.

(iv) Import of genetically altered rodents – to allow genetically altered rodents to be obtained from recognised scientific establishments outside the United Kingdom for use under this project licence.

(v) Assessment of arthritis (motion analysis) – to allow motion analysis studies in rodents.

2. Why the changes are needed:

(i) Additional protocol 19b 6 (experimental osteoporosis) – Osteoporosis was not a primary focus for our research until very recently. As a consequence of our work using inflammatory models of arthritis we may have discovered a receptor with crucial functions in bone. This discovery may have extremely important implications with regard to our understanding of diseases such as osteoporosis as well as rheumatoid arthritis. Both these diseases weaken our bones and make them more likely to fracture. As a result of our in vitro studies we found that this receptor called Death receptor -3 controlled the generation of osteoclasts, the only cells in our body able to destroy bone. Thus Death receptor 3 could well control bone growth, quality, strength and repair tipping the bone balance away from health and towards disease. We think that Death receptor -3 could prove to be an important target for therapy in osteoporosis and a marker for patients who go on to develop bone fractures. Our studies in a rodent model of osteoporosis will provide proof of concept data to support the role of death receptor 3 in osteoporosis and complement the data that we will obtain from human tissue specimens.

(ii) Permission to use novel imaging techniques to assess arthritis – We have access to new in vivo imaging facilities at Cardiff University for non-human research. These include: Magnetic Resonance Imaging (MRI), Magnetic Resonance Spectroscopy (MRS), Computer Tomography (CT) and Positron Emission Tomography (PET). Implementation of these high-resolution imaging techniques for the assessment of disease activity and associated brain function present an opportunity for us to significantly reduce the number of animals used for our research in the long-term.

(iii) Change from mouse to rodent (19b protocol 1, 2 and 5) – We were recently awarded a £2.5 million grant from the arthritis Research Campaign to establish a Centre of Excellence in Bioengineering and Biomechanics Research. We will use rats for our developing mathematical models of animal movement (a separate licence application will be submitted for these studies). Mice are likely to be too small for fitting motion sensors, therefore accurate measurement of mathematical parameters would be compromised which may preclude the development of these models. We aim to perform complimentary studies in arthritic rats to understand the effects of biomechanical forces upon inflammation, pain and arthritis progression.

(iv) Import of genetically altered rodents – genetically modified rodents from overseas collaborators may become available for investigation during the course of this project.

(v) Assessment of arthritis (motion analysis) - Our current assessment criteria for rodents with arthritis incorporate measures of inflammation

and joint damage (using tissues excised at termination). Our current assessment criteria do not provide quantitative in vivo functional data pertaining to biomechanical forces through joints or animal behaviour (e.g. ability to walk). We would like to use motion analysis as a method of refined functional assessment applicable to experimental models of arthritis. This method is sensitive to impaired movement (induced by arthritis) and recovery (induced by arthritis therapy). Motion analysis also offers valid representations of relevant aspects of human disability caused by arthritis.

3. Species and number to be used

(i) Additional protocol 19b 6 (experimental osteoporosis) – 500 additional Adult wild-type or genetically altered Mice or Rats in total to be used in the one new protocol (Protocol 6) over the remaining two years of the licence.

(ii) Permission to use novel imaging techniques to assess arthritis – An additional procedure which may be applied in Protocols 1, 2, 3, 5 and 6. No additional animals requested.

(iii) Change from mouse to rodent (19b protocol 1, 2 and 5)- No additional animals requested

(iv) Import of genetically altered rodents)- No additional animals requested

(v) Assessment of arthritis (motion analysis) - No additional animals requested

4. Effects on the animals:

a) Additional protocol 19b 6 (experimental osteoporosis) – Bone mineral density (BMD) will be measured in ovariectomised mice and rats and sham operated age-matched controls. When BMD falls by 30% versus baseline values or 45 days after ovarectomy (whichever happens first); the experiment will end. Tissues and blood will be harvested for additional experimental outputs. BMD, body weight, femur length and indices of bone structure (by micro-computed tomography (microCT)) will be measured at end-point together with serum markers of bone resorption and bone formation. Lumbar vertebrae and distal femora will be subjected to dynamic histomorphometric assessment. Novel sensitive imaging techniques may be applied eg magnetic resonance imaging to determine BMD and bone morphology in longitudinal studies; thereby reducing animal usage.

This procedure is usually very well tolerated by animals. Aseptic surgical technique will be used to greatly reduce the risk of infection. Complications, which occur in approximately 1% of cases, consist of wound infection and dehiscence. Wound sites will be inspected daily following surgery. If a localised abscess is present, or wound dehiscence has occurred due to loss of a clip, it may be possible to deal with this as a simple procedure under a further anaesthetic, otherwise the animal will be killed. Animals will be assessed at least twice each week after the wound has healed. Animals showing adverse reaction will be observed more frequently. Examples of adverse reactions include hunched posture, piloerection, and lethargy.

b) Permission to use novel imaging techniques to assess arthritis – All imaging will be conducted under general anaesthesia, with concurrent physiological monitoring, both with and without concurrent delivery of pharmacological agents, drugs and subcutaneous electrode stimulation depending on method and the individual experiment. In most experiments, and certainly in the early development and validation of each technique, the animals will be killed at the end of the scans while still under anaesthesia. Once each method is established, and when the animals' health and welfare is known not to be significantly compromised, some studies will by conducted under anaesthesia with recovery in order to allow longitudinal and other assessments within the same animal. This will significantly reduce animal usage longterm.

c) Change from mouse to rodent (19b protocol 1, 2 and 5)- No effect on animals.

d) Import of genetically altered rodents)- No effect on animals

e) Assessment of arthritis (motion analysis) - Movement characterisation is achieved by three dimensional video motion analysis. To achieve accurate location of body parts and joints, small markers (either adhesive or paint dots) are applied to fur or skin. Skin may be shaved for better contact of markers, which will be undertaken under sedation or light general anaesthesia (for restraint) to minimise stress to the animal. In order to assess and quantify behaviour/mobility animals will be place on a narrow raised beam (60 cm above ground). The ability of the animal to walk across the beam to a safety platform will be monitored. Animals occasionally falter when crossing the beam and can, on rare occasions, fall. If the animal falls from the beam; testing will be stopped. If the animal does not show an efficient righting response then the animal may be killed. In general motion analysis assessments will take place in conjunction with arthritis swelling measurements and finish at the endpoint of individual experiments which will not exceed defined end-point for specific experimental protocols.

5. Brief summary of the cost-benefit ratio of the proposal:

1. Costs to the animals:

a) Additional protocol 19b 6 (experimental osteoporosis) – 500 mice or rats will experience a mild surgical procedure which is well tolerated

b) Permission to use novel imaging techniques to assess arthritis – mice and rats infrequently experience ill health from the administration of radiation administered/delivered as the doses fall below those that might cause harmful effects

c) Change from mouse to rodent (19b protocol 1, 2 and 5) –No cost implications to the animals

d) Import of genetically altered rodents- No cost implications to the animals

e) Assessment of arthritis (motion analysis) - Small markers (either adhesive or paint dots) applied to fur or skin, sedation or light general anaesthesia will be used for restraint to minimise stress to the animal. On rare occasions animals can fall when crossing the beam. If an animal falls

from the beam testing will be stopped and if the animal does not show an efficient righting response then the animal may be killed.

2. Potential benefits:

This work will provide additional data to address Objectives 2 and 4 of the PPL, thus providing a valuable contribution to our knowledge of the functioning specific genes, cells and mediators in the muskuloskeletal system in the rat and mouse. The data generated will have potential usefulness in improving our understanding of musculoskeletal disease in humans.

3. How the benefits outweigh the costs:

In order to obtain data streams in line with our objectives it is necessary that animals develop osteoporosis. Animals are unlikely to experience functional impairment as a result of osteoporosis induction; this would therefore be classed as a mild procedure. Any adverse effects, which may occur infrequently, are readily controlled by regular assessment and appropriate intervention. Rat and mouse both provide excellent model systems in which to study the relationship between the immune system and osteoporosis development.

Osteoporosis is a disease characterised by low bone mass which leads to enhanced bone fragility and increase in fracture risk. By association with fragility fractures, osteoporosis presents as a major public health challenge, which is likely to become more problematic by virtue of the fact that as we get older our bone mass declines and the risk of fractures increases. Osteoporosis, is therefore becoming increasingly prevalent with the aging of the world population and despite the availability of preventative and therapeutic agents, the social and economic burden of osteoporosis is growing steadily. This highlights the requirement to identify molecules that precipitate loss of bone mass, obtain a greater appreciation of the mechanisms by which these molecules regulate bone turnover and to utilise this information to develop alternative novel targeted therapeutics for osteoporosis. The complex genetic, cellular and molecular basis for normal skeletal development has not been unravelled. Advancement of our understanding is unlikely from clinical studies alone because of heterogeneity and our limited ability to intervene in the genetics, personal environment or skeletal biology of human subjects. Studies in animals are justified as this is the only means by which we can gain important additional knowledge concerning this disease.

Refinement of our assessment criteria for arthritis by implementing novel imaging and motion analysis techniques provide important additional information regarding the aetiopathogenesis of arthritis; these procedures will not cause pain, distress or discomfort to the animal.

All experimental procedures are very closely controlled and all animal work is always undertaken at the highest professional standard in collaboration with the named veterinary surgeon and animal care advisors.

Amendment 5 – Immune responses during persistent virus infection

Lay summary:

1. What the changes are:

Addition of protocol 19b 1, subsection 5. This will enable us to administer antibiotics to animals to eradicate low-grade bacterial infections that might otherwise influence some of our results.

Addition of protocol 19b 1 subsection 6. This will allow us to measure the lung function of MCMV-infected mice

Alteration of protocol 19b 1 subsection 4. This will enable us to increase the amount of blood that we take from mice in some of our experiments.

Addition of protocol 19b 1, subsection 1d. This will enable us to administer brefeldin A to mice in some experiments.

The addition to protocol 19b 1, subsection 5 is required to enable us to administer antibiotics to some mice. Experiments in our laboratory have shown the possible importance of a protein called Interleukin-22 (IL-22). Our data suggests that IL-22 may promote antiviral immune responses. It is therefore important to understand how and where IL-22 is produced during virus infections. However, bacteria that colonise healthy mice can actually switch on IL-22. Therefore to understand whether components of the immune system can directly induce IL-22 (and therefore represent possible drug targets to help treat virus-infected patients), we need to treat some mice with antibiotics prior to and during experiments to ensure that resident microflora are not skewing the results from our studies.

The addition of protocol 19b1 subsection 6 will enable us to measure lung function of MCMV-infected mice. Our laboratory has recently shown an important role for several proteins of the immune system in controlling pulmonary cytomegalovirus infection. Given that cytomegalovirus pneumonitis is a deadly disease in humans, it is important to understand how these proteins: a) limit virus growth in the lung and b) protect lung function during infection. Measuring the respiratory function of mice during experiments will therefore help us answer the latter question.

The alteration to Protocol 19b 1 subsection 4 will enable us to take more blood from mice in certain experiments. We have developed new technology which enables us characterise individual white blood cells. This technology, which is called T cell receptor clonotyping, will enable us to characterise what immune responses afford the best protection against viruses. We will do this by using TCR clonotyping to characterise immune responses triggered by vaccination, and then tracking these immune responses in the same mouse before and after subsequent challenge with virus. We will then determine what types of vaccine-induced immune responses correlate best with protection from infection. Because these experiments will track immune responses in the same mouse over time, we need to bleed mice to obtain white blood cells at the different time points. To obtain enough white blood cells to do TCR clonotyping analysis, we need to take up to 20% of their total body volume at each time-point.

The addition of Protocol 19b 1 subsection 1d will enable us to administer brefeldin A to MCMV infected mice. A major aspect of our research is studying T cell responses to MCMV, in particular the production of antiviral cytokines by these cells. Following synthesis, cytokines are transported out of cells, thus are most commonly measured extracellularly in serum or cell supernatants. To identify particular cell types that express cytokines (e.g. MCMV-specific T cells) in vitro

techniques are employed whereby T cells are specifically stimulated in medium containing inhibitors of protein transport (such as brefeldin-A) which result in the accumulation of cytokines intracellularly allowing their detection within cells by flow cytometry. There are at least two problems with this approach. Firstly, stimulation of the cells in vitro may alter their behaviour thus cytokine expression measured in vitro may not accurately reflect in vivo activity. Secondly, stimulation of cells in vitro may result in activation induced cell death of T cell subpopulations thus valuable information relating to in vivo activity may be lost. Methods for inhibiting protein transport in vivo have been developed to counteract these problems. In vivo administration of Brefeldin-A results in accumulation of intracellular cytokines allowing cytokine-producing cells to be detected ex vivo without any need for in vitro manipulation (Liu F, Whitton JL. Cutting edge: re-evaluating the in vivo cytokine responses of CD8+ T cells during primary and secondary viral infections. J Immunol. 2005 May 15;174(10):5936-40.) This approach represents a significant technical improvement to current in vitro methods as the data produced reflect more accurately in vivo activity, thereby increasing the value of the data obtained through use of the animal model.

2. Species and number to be used

The proposed procedures will be utilised to extract the maximum information from existing studies. Therefore, no extra mice will be required.

3. Effects on the animals:

Addition of protocol 19b 1 subsection 5.

Administration of antibiotics to mice will not cause adverse effects. This will be performed on no more than 100 adult mice.

Addition of protocol 19b 1 subsection 6.

Minor stress will be caused to the animals due to transfer of mice into the machine. After the lung function is measured, mice will be killed by schedule 1. This will be performed on no more than 300 adult mice.

Alteration of protocol 19b 1 subsection 4.

The increased volume of blood taken from animals will potentially cause anaemia. However, we will administer saline to the animals after each bleed, thereby alleviating this possible problem. This will be performed on no more than 600 adult mice.

Addition of protocol 19b 1, subsection 1d

The dose of Brefeldin A which is needed for visualising the production of secreted mediators is a maximum of 20mg/kg (maximum 0.5 mg per mouse). At this dose, no deleterious effects have been observed (Liu and Whitton, 2005). Indeed, in a mouse model of amyloidosis, Brefeldin-A has been administered to mice at 0.5mg per mouse daily for 5 days with no deleterious effects (Stenstad and Husby, 1996). Therefore we do not expect to observe any adverse effects. Furthermore, mice wil be killed by schedule 1 killing 5-6 hours after injection of brefeldin A. This will be performed on no more than 600 adult mice.

4. Brief summary of the cost-benefit ratio of the proposal:

1. **Costs to the animals:**

No more mice will be required for these studies. Instead, we will gain more information from each experiment (therefore from each mouse).

In some experiments, antibiotics will be given in the drinking water of mice. This will not cause any distress or discomfort to the mice. In experiments where we will study lung function, mice may experience mild stress due to transfer into, and restraint within, the plethysmography equipment. Mice will only be in this equipment for a short period of time.

Taking larger volumes of blood from some mice would potentially cause anaemia. However, we will also administer fluid to the animals after each bleed. Therefore mice will have an additional intraperitoneal injection after each bleed.

Injection of brefeldin A will cause only minor discomfort to the animal.

2. **Potential benefits:**

The treatment of mice with antibiotics in some experiments will enable us to rule out the possibility that endogenous bacteria are influences our results. Therefore, it will enable us to further define the immune mechanisms that induce the production of important antiviral molecules. These studies potentially have large implications for design of antiviral vaccinations and immune therapeutics.

Understanding the mechanisms that protect the lung from damage during virus infections also has major implications for the design of any vaccine that protects the lung from infection. Therefore, studying the influence of immune molecules on lung function during MCMV infection will provide valuable information for future vaccines.

The ability to track individual white blood cells over time in the same mouse will revolutionise our research, enabling us to gain massive amounts of detailed information regarding the immune responses that are induced by antiviral vaccines.

For most experiments utilising MCMV, groups of 3-4 mice are routinely used. However, due to the technical difficulties associated with in vitro cytokine analysis (see above), groups of 4 – 6 mice are currently used for this type of work. Often experiments require repeating on at least two separate occasions. We anticipate that this new approach will represent a significant improvement in the detection of biologically relevant T cell responses thus it is likely that use of this technique will reduce the number of mice used for these studies to 3 mice per group with experiments being repeated once only. No additional mice will be required for this work.

3. **How the benefits outweigh the costs:**

Treatment of mice with antibiotics, and the measurement of lung function both cause minimal stress and harm to the mice. In contrast, the information that we will gain from these experiments could have potentially massive implications for vaccines and other therapies that protect us from respiratory infections. Furthermore by counteracting the potential adverse effects of

additional bleeding, we will obtain a large volume of informationfrom our TCR clonotyping experiments whilst causing minimal harm to the mice.

Following brefeldin A injection, mice will be frequently by animal care advisors and the researchers involved in the work. As with all procedures on this licence, staff will be trained to a high standard in techniques of animal restraint thereby minimising distress to each individual animal. This technique will enable us to measure biologically relevant molecules associated with protective immune responses during viral infections using fewer mice per experiment. Therefore, the benefits of this experiment greatly outweigh any adverse effects.