

HOME OFFICE LICENSING

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

Applications:

Project Title: Induction of Anti-Viral Immunity

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.

Project Title: Studying Arthritis Pathology and Assessing the Impact of Therapy

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.

Project Title: Anti-microbial immune responses and inflammation

An application to continue work from an existing Project Licence. The application has completed ERP and is being prepared for Home Office submission, and the project abstract is reproduced below for further information.

Amendments:

Project Title: Parasitic Infections of Fish

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.

Project Title: Mechanisms of plasticity in 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.

Project Title: Regulation of Peripheral Receptors for Biogenic Amines

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.

BSO
10 Jan 2012

LAY SUMMARY AND ABSTRACTS FROM APPLICATIONS PROCESSED BY CHAG

Please NOTE - This is for information only - no assessment by the Committee is needed

Application 1 - Induction of Anti-Viral Immunity

Abstract. A critical goal for vaccines is to induce long-lived, or “memory”, immune responses that protect us against infections. Current influenza vaccines induce antibody immune responses that target proteins on the outside of the virus. However, influenza can alter these proteins and vaccines require annual production to keep up with these changes. Furthermore, vaccine-induced antibody does not protect against multiple influenza strains and will likely have little impact in the event of a new pandemic outbreak. T cells are immune cells that can recognise proteins on the inside of viruses. Because influenza cannot tolerate changes in these proteins the virus cannot hide from T cells, and because these inner proteins are conserved between influenza viruses, T cells can afford protection from multiple, including pandemic, strains. The overall aim of this project is to design and test new vaccine strategies based on conserved proteins. At present however, there are no methods available for replicating an intact immune system using laboratory-based methods. The reason for this is that the immune system comprises a complex network of different cell-types that interact within organised structures (lymphoid organs) in the body. Different components of the immune system may behave differently once removed from this environment. It is essential therefore that the effect of a part of the immune system is tested when these interactions are intact.

The animal model to be used is a mouse model as over the past few decades, a large body of information has been gathered about the mouse immune system. Much of this information has shown that immune responses in mice closely parallel those in humans. A typical experiment would involve infecting a mouse with influenza virus and determining which components of the immune system are essential for allowing the mouse to mount an adequate immune response to the virus. An adequate immune response is considered a response that clears the virus, does not cause damage to tissues and organs and which enables the mouse to develop long-lived immunity to the virus. Infection of mice with influenza virus is well characterised in terms of adverse effects. The severity of such effects will not exceed a short (24 hour) period of listlessness and in a minority of cases, some weight loss. The procedures involved in this study are well established and can therefore be conducted in a manner that provides maximum information but minimal distress to the animal. Several similar experiments have been carried out previously and have provided sufficient information to enable us to calculate the minimum group sizes required to achieve statistical significance.

In summary the main aim of this work is to identify factors that allow induction of an adequate immune responses capable of clearing a virus infection but which does not cause damage to normal organs and tissues. The mid- to long- term goal of the work is to provide information that can be used in the design of anti-influenza virus vaccine strategies to be used in humans.

Application 2 - Studying Arthritis Pathology and Assessing the Impact of Therapy

Abstract. Arthritis is a common painful disease that causes inflammation and destruction of the joints. This in turn makes patients miserable, causes disability and in some cases death. Arthritis affects young people as well as the elderly. In this project, we will look at the way in which inflammation is triggered and the ways in which inflammation can cause breakdown of the structure of the joint. To date a cure for arthritis has not been developed; in understanding the way by

which the joint becomes damaged we are better placed design new, more effective medicines to treat arthritis and prevent disability.

Not that long ago it was believed that drugs that are used by doctors to treat arthritis made little, if any, difference to the crippling effects of arthritis in patients. The reasons for this were as follows:

- (i) it was difficult to see patients early enough to start treatment. In fact by the time specialist doctors called Rheumatologists came to see patients in hospital based clinics their joints were already damaged and could not be repaired ,
- (ii) an accurate diagnosis was not possible and
- (iii) therapies were ineffective.

Developments in arthritis research over the past 15 years have taught us that we should aim to switch off inflammation in patients presenting with arthritis as early as possible. One the whole this sounds like a reasonable plan, but, How early is 'early'? and Which early inflammatory signals should be targeted?

These questions will be addressed in this project.

In achieving the projects objectives we will:

- (i) Learn about the way that cells in the blood communicate with cells within the joint and learn more about how arthritis develops.
- (ii) Cells are ordered into action through release of communication proteins, called cytokines. This occurs through the cytokine binding to specific 'receptor' proteins on a cell surface. The complex series of events that follow contribute to disease progression. Identifying the consequence of this dialogue and understanding how cytokine activities are regulated will lead to the identification of agents beneficial in arthritis treatment.
- (iii) Control of cytokine action occurs in several different ways. One way is through release of cytokine-specific receptors from cells that mop up free cytokines. This typically dampens the effect of the cytokine but can enhance the properties of the cytokine it binds. Studies will determine the disease process regulating receptor generation and establish its significance in arthritis.
- (iv) Other signals, some that are known and others that have not yet been discovered control inflammation. Understanding how these molecules control inflammation and joint damage is necessary before we find a cure for arthritis.

Clues as to how arthritis develops reside in the pre-clinical stage (where symptoms may not be apparent to the patient) and where the initial triggering and induction events that lead to arthritis development take place. Unfortunately, this phase of human arthritis is not readily accessible to investigation and therefore remains speculative. The spectrum and the progression of arthritis is controlled by the immune system, genetic and environmental factors, therefore, a better understanding of the immune mechanisms during the early phase of arthritis is critical to the development of new therapies that may one day lead to a cure of the disease. In vivo models of arthritis therefore serve as essential tools to investigate the underlying mechanisms of early, intermediate and late stages of

disease. With advances in molecular biology, immunology, bioinformatics and drug design techniques, the possibility of developing novel therapies for arthritis is all the more promising.

Wherever possible hypotheses are tested rigorously in vitro using model systems, that we have established in our laboratory, such as cell lines (fibroblast-like synoviocytes (FLS), chondrocytes, leukocytes and osteoclasts), synovial tissue explants (human, bovine and murine) and clinical samples (blood, serum and synovial fluids) originating from patients with rheumatoid arthritis, osteoarthritis or volunteers unaffected by arthritis. Definitive studies are then carried out in vivo on a small number of animals (usually 3) using an appropriate model. Tissue and blood samples are collected routinely at the end of the experiment. These will be used to look at markers of inflammation and joint destruction within the joint and in the circulation. If initial results look promising then experiments will be repeated giving an indication of reproducibility. In each case the minimum number of animals that gives statistical significance will be used.

The procedures involved in this study are well established and can therefore be conducted in a manner that provides maximum information but minimal distress to the animal. Several similar experiments have been carried out previously and have provided sufficient information to enable us to calculate the minimum group sizes required to achieve statistical significance. This experience has also allowed us to define early end-points for experimental protocols that involve the induction of arthritis and thereby keep animal suffering to a minimum. Furthermore, analgesics are routinely administered early to avoid the symptomatic pain associated with arthritis development.

All animals are given environmental enrichment (chew toys, tunnels) and are allowed to express normal behaviors by being kept in clan groups where possible, provided with bedding to shred, and food treats such as sunflower seeds. Veterinary staff are also always accessible for advice and assistance in matters pertaining to the welfare of the animals. Rodent models of arthritis have been developed in both rats and mice. Other species have also been used over the years, however rodent models are most common, due to cost, homogeneity of the genetic background, and in mice, the capacity to use genetically modified strains. Much of this information has shown that immune responses in mice closely parallel those in humans.

In summary the main aim of this work is to determine the true impact of the immune system upon arthritis and to determine whether this activity can be harnessed for treatment purposes.

Application 3 - Anti-microbial immune responses and inflammation

Abstract. There is still a great need for therapeutic interventions that promote protective immune responses against pathogens, but these responses must be measured to limit tissue damage and retain normal tissue function, particularly during chronic conditions.

Our aim is to determine factors that control the balance between protective host defence, inflammation and immunity to re-infection and the resolution of inflammation and control of normal tissue function. By better defining the cells and cellular mechanisms involved in the maintenance of tissue function and the development of protective immunity we aim to explore the therapeutic potential of the manipulation of normal and immune processes to aid in the resolution of infection and the restoration of tissue function with limited tissue damage.

We will dissect experimental mouse models of infection and inflammation as well as examine the normal physiological processes that occur within tissues. The complex nature of the microanatomy of the immune system and the tissues themselves necessitate the use of animal models and the mouse is the most appropriate species of the lowest degree of neurophysiological sensitivity. Additionally, mice have to be used because of their amenability to genetic modification and the need for genetically modified mice during this project.

The experimental models are well established and individual experiments will be designed with the aid of appropriate statistical analyses to ensure that no more animals are used than required for statistical validity. The majority of protocols are simple and mild involving only transient discomfort, usually after a single injection. Some protocols will require a greater potential for discomfort, such as those involving infection with live microorganisms. For these 'moderate' protocols a detailed scoring system will be used to assess the welfare of the animals and humane endpoints will be used to prevent additional adverse effects. A small percentage (~5%) of protocols will involve bone marrow transplantation and these will be managed with prophylactic antibiotics to limit the potential for additional complications. It is estimated that approximately 4000 mice will be used during the 5 year course of this proposal, which reflects the natural expansion of a successful research programme.

This programme of work is primarily centred around the burden of infectious disease and the tissue dysfunction that occurs as a consequence. The project is designed to examine broad aspects of tissue function and immune responses from normal development, immune challenge and the restoration of normal tissue function. This programme of work, supported by a previous project grant generated approximately 20 peer-reviewed manuscripts on immune function and attracted an additional seven grants were won during this period. Many of these outputs were primarily directed towards the manipulation of immune cell activity for therapeutic gain. Several of the publications are internationally-leading in the field and at least two of them were developed with the 3Rs, particularly replacement, as a specific objective.

This work will continue to advance our understanding of fundamental mechanisms of tissue function and immunity and aims to open novel avenues for therapeutic exploitation.

Amendment 1

Lay Summary: Parasitic Infections of Fish

1. What the changes are:

Two additional procedures to allow the collection of blood samples from fish, under the existing Protocol 2:

- i) Withdrawal of blood from the caudal vein.

This is a standard way of taking blood samples from fish (www.ccac.ca/Documents/Education/DFO/4_Blood_Sampling_of_Finfish.pdf, www.uoguelph.ca/~aqualab/forms/fishbloodsamplingSOP.pdf) that will be done either under anaesthesia with recovery (AB), or under terminal anaesthesia (AC). For small fish (such as *Gasterosteus aculeatus*), a large enough sample will only ever be possible without recovery, due to the limited total blood volume of the animal (and the difficulty of accessing the caudal vein without damage to

surrounding tissues). With larger fish, a small blood sample can be obtained under AB.

ii) Withdrawal of blood by cardiac puncture.

This technique is necessary for very small fish (all *Poecilia reticulata*), to get a sufficient sample, and will always be carried out under terminal anaesthesia (AC).

2. Why the changes are needed:

These non-surgical procedures will support our existing research program. Specifically, they will allow us to measure levels of the stress hormone cortisol, for example, that are circulating in the blood plasma of fish. This enables us to validate the new protocol we are developing to measure hormones remotely from fish holding water (fish excrete hormones across their gills into the water, at levels determined by those circulating in their blood plasma).

Since submission of the original licence, a new grant was awarded that will utilize the 'hormone in water' technique: It will investigate the links between the hormonal and behavioural profiles of animals, and how these relate to disease susceptibility and transmission. The grant provides specific funds to carry out this technique in a wide range of experiments (not foreseen in the original application). Because we will be using the method for more fish, and in a wider range of contexts, validation will increase the weight of our results. Thus, by proving the relationship between circulating hormones and hormones in water, we will be able to accurately estimate circulating levels of hormone in the future without taking a blood sample. In contrast, remote measures without this validation are useful, but they are relative, because we cannot directly relate them back to circulating levels.

3. Species and number to be used

No additional animals will be used; 50-100 of the fish already covered by the existing PPL Protocol 2 will be used.

4. Effects on the animals:

Animals sampled under terminal anaesthesia will not be affected by the actual procedure. Animals sampled via the caudal vein will be under anaesthesia during blood collection. This will minimise the stress of sampling, and increase the ease of sampling, thus increasing accuracy of placement of a fine gauge needle into the vein. As stated in the PPL, rarely (ca. 1:1000 for species that we use most frequently), an animal will not recover (die) from anaesthesia.

Chances of bleeding after the sample is taken are very low - a very fine gauge needle will be used. Chances of infection after the sample are also low, and are no higher than for Visible Implant Elastomer (VIE) marking s38 Health and Safety which is already allowed under this PPL. In both cases, care is taken to monitor recovery of fish from anaesthesia, and to determine that there are no side effects of the procedure. Rarely, fish may obtain bacterial or fungal infections at injection sites. However, this is closely related to fish health and water quality, and has never occurred in this lab. If it did occur, fish would be treated immediately with the appropriate anti-fungal or anti-bacterial treatment and monitored to ensure full recovery.

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

a) Costs to the animals:

50-100 fish used for blood sampling via the caudal vein will experience only transient discomfort from the placement of the needle, after they recover from anaesthesia. The amount of blood (<5 µl) removed is not sufficient to itself cause physiological problems for the fish.

b) Potential benefits:

Blood sampling allows direct measures of circulating hormone levels. These are needed to biologically validate the remote hormone collection technique, from water that fish have been held in. (Assay validations are carried out during every analysis to ensure repeatability of sample measures, and comparison between samples). Biological validation allows us to directly relate circulating levels to excreted levels of hormones. This allows us to give accurate estimates of circulating levels (which influence physiology), rather than comparing relative levels from excreted samples. Remote hormone measures provide an invaluable way of quantifying animal physiology without any invasive sampling.

As such, it is an important tool for refinement, as described under the 3Rs. From a research point of view, remote hormone samples are less stressful for the fish, which is important as additional stressors affect the production of stress hormones, and hence behaviour and potentially the course of parasite infections (both of which we studying). Reducing stressful procedures therefore will increase the value of our experimental results.

c) How the benefits outweigh the costs:

As noted above, the blood collected will allow us to validate a technique that will allow us to collect data on hormone profiles with minimal disturbance to fish. This will greatly increase the range of questions we can ask about fish that are already being used in our experiments.

Adverse effects will be minimised by (a) anaesthetising the fish prior to sampling, and (b) taking terminal samples from fish too small to easily give a sample under anaesthesia with recovery. Work will only be carried out by staff fully trained in the technique. Fish sampled will be closely monitored during recovery to ensure there are no long term effects of the procedure. As noted above, this procedure is no more stressful for the fish than having VIE markings placed intra-muscularly for identification purposes.

Amendment 2

Lay Summary: Mechanisms of plasticity in the visual cortex

1. What the changes are:

Inclusion of an additional Ca^{2+} biosensor (a calcium sensitive molecule that makes electrical activity of neurons visible for brain imaging) which is to be delivered to neurons by viral infection

2. Why the changes are needed:

The changes are needed in order to improve the labelling of individual neurons for chronic functional imaging. Extensive studies in the labs of my

collaborators s40 Personal Data, s38 Health and Safety have shown that the Ca^{2+} biosensor TN-XXL that I had originally proposed to use for chronic single-cell brain imaging does not work as well as previously thought. A new Ca^{2+} biosensor has recently become available, called GCaMP3. A change to the licence is needed because GCaMP3 (unlike TN-XXL) is not inherited. It has to be inserted into neurons via a so-called vector, i.e. by combining it with a virus that is injected into the target brain area and infects neurons locally.

3. Species and number to be used

No change in the species (mouse) and number of animals.

4. Effects on the animals:

Animals need to be injected 1-4 weeks prior to the assessment with the viral vector; this involves surgery under general anaesthesia with subsequent recovery. A maximum of 320 animals are expected to undergo this procedure (100 for Aim 3, 60 for Aim 4, 60 for Aim 5, 50 for Aim 6, 50 for Aim 7). Both the biosensor and the viral vector are harmless to the animals.

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

d) Costs to the animals:

The animals that are to express GCaMP3 will undergo an additional surgical procedure with recovery. However, the lab of the applicant has many years of experience with this type of procedures which should help keep the costs to the individual animals involved to a minimum.

The viral vector does not contain pathogenic genes and will not bestow any disabling mutations on the animals or endow them with any harmful properties. Non-human primates, rats and mice have been successfully injected with this vector with no adverse effects on the animal's health or welfare. Since the viral vector does not contain genes needed for replication (i.e. for multiplying and spreading) transmission through the host will not occur. The vector is unable to spread from the brain, so will not be excreted. Therefore, although post-operative animals will be housed individually, they will not represent a threat to other animals housed in the same animal facility. The injection itself may cause a small amount of localised inflammation which can be controlled by giving an anti-inflammatory drug.

e) Potential benefits:

The functional brain images obtained with GCaMP3 will be of much better quality and can be obtained over a longer period of time compared with TN-XXL. This, combined with lower toxicity, means a higher success rate of experiments, and therefore potentially a lower number of animals.

f) How the benefits outweigh the costs:

The improved data quality and lower toxicity of the new calcium biosensor increases the likelihood of achieving the objectives of the project, using fewer animals, and thus outweighs the increased costs to the animals resulting from the additional surgical intervention.

Amendment 3**Lay Summary: Regulation of Peripheral Receptors for Biogenic Amines****17b Objective 1:**

Ureaplasma urealyticum, a species of mycoplasma organisms, is a sexually transmitted organism which can cause pneumonia in premature neonates. Ureaplasma urealyticum infection in the neonate occurs secondary to maternal transmission and very low birth weight neonates with Ureaplasma urealyticum pneumonia are approximately two times more likely to develop chronic lung disease (COPD) and asthma (Gannon, 1993). We wish to extend our studies to new-born guinea-pigs to mimic this syndrome by exposure to bacterial LPS and to determine whether the new-born also show increased reactivity to biogenic amines.

18a Outline of the programme of work

Immature guinea-pigs will also be used to measure airways hyperreactivity.

18b Objective 1: Neonatal chronic respiratory disease will be modelled in immature guinea-pigs since this species is able to be independent of its mother and to be freely mobile for measurements of airways function in unrestrained free-roaming animals almost immediately after birth. We will breed these animals in-house to avoid any travel-induced distress of pregnant females or risk of loss of mothers or offspring. To preserve numbers we will use all offspring (males and females).

Numbers of immature guinea-pigs added to 19a Immature guinea-pigs 60

Immature guinea-pigs specified in 19b Protocol 1

Lay summary

Premature babies from mothers carrying infections from particular strains of bacteria are often born with pneumonia and go on to develop chronic lung disease and asthma. To characterize the changes to the airways associated with bacterial infection and to determine whether it is associated with increased sensitivity of the airways to bronchoconstriction, we will expose new-born and immature guinea-pigs to bacterial lipopolysaccharide that mimics bacterial infection. We chose the guinea-pig as it is independent of its mother soon after birth and is mobile and free-roaming to permit measurements of respiratory function in the unrestrained and unanaesthetized animal. While this contrasts with the dependence of human babies for many months after birth, the respiratory systems of new-born humans and guinea-pigs are at a similar level of development. We will breed these animals in-house to avoid any travel-induced distress of pregnant females or risk of loss of mothers or offspring. To preserve numbers we will use all offspring (males and females).