Home Office

NON-TECHNICAL SUMMARY

Mice with adaptive human immune systems and their application for drug and vaccine discovery

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
    - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Antibody drug, Vaccine, Cancer, Covid 19, T cell

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The purpose of this project is to genetically alter mice to humanise their immune system and subsequently use such mice to identify and develop novel drugs for treating diseases and vaccines for disease prevention in humans.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is important to undertake this work so that we can discover new drugs and better vaccines for treating and preventing many different diseases in people.

Mice with human immune systems respond to vaccination just like people do. Therefore, by testing different vaccines in mice with human immune systems we can identify better vaccines that are much more likely to work in people.

We can also isolate human antibodies from these mice. These can be developed as drugs to treat diseases like those caused by coronaviruses such as Covid 19 or cancers like leukaemia and prostate cancer.

What outputs do you think you will see at the end of this project?

New medicines, namely vaccines and drugs.

- Drugs that offer potential new treatments for a form of leukaemia.

- Vaccine candidates that offer improved protection against malaria

- A second generation Covid 19 vaccine, improving on the proportion of vaccinated people who become immune to the virus following vaccination and improving on how long this lasts.

Basic Science benefits:

- Publications in the peer reviewed scientific literature

- Genetically engineered mice that can be widely used by the scientific community to pursue related projects in other disease areas.

- Grants to support continuity of the work for an additional funding period.

Who or what will benefit from these outputs, and how?

- All countries of the world could benefit from the discovery of new information that supports the development of vaccines for Covid 19.

- Societies in countries where malaria is endemic could derive benefit (in the long term) if we are successful in identifying a better vaccine for malaria, recognizing that it can take many years for a result in the laboratory to be shown to provide long term benefit in humans.

- Society will benefit from knowledge generated as this provides better understanding of diseases and methods to treat and/or prevent them.

- Research institutes, pharmaceutical companies and non-governmental organisations will be able to take information produced by such studies and use it to support the development of drugs or vaccines that ultimately provide benefit to patients.

How will you look to maximise the outputs of this work?

We will collaborate with experts in the field, for instance our work on vaccines is a collaborative endeavour with a variety of laboratories.

We will publish scientific papers, thereby disseminating knowledge.

We will file patents, thereby placing in the public domain detailed knowledge of the discoveries we have made.

We will raise money so that the development of promising medicines can continue.

We will out-license promising drugs in situations where we cannot afford to develop these drugs ourselves, thereby allowing the benefits to emerge.

Species and numbers of animals expected to be used

- Mice: 38,550

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The mouse was selected for this project because of the availability of advanced technologies to make "transgenic" mice. Transgenic mice are mice in which their genome has been genetically altered. This is accomplished by adding or removing genetic information at very early embryonic stages. The methods used to do this are very efficient and extensive changes can be made. The refinement of these techniques mean that many fewer animals are needed compared to achieving the same goals in another species.

The immune system of the mouse is similar to that of humans so we can replace mouse genes with the equivalent human ones to make mice that have a more "human relevant" immune system. Although very large stretches of mouse DNA has been replaced with the human equivalent the human genes substitute for the mouse ones. The resulting mice do not exhibit any signs of suffering or distress. The other genetic changes we will make in this project involve removing genes for drug targets from the genome, which should not adversely affect the mice either. Adult mice are used for vaccinations because their immune systems are mature.

Typically, what will be done to an animal used in your project?

To produce transgenic mice fertilised embryos are collected which are manipulated under the microscope. These are transferred to foster mothers who become pregnant, give birth and rear their pups.

The embryo transfer is done under general anaesthesia as we need to make a small cut in the skin to place the embryos in the uterus or oviducts. Typically, the mice recover very quickly from surgery, they are actively running around, eating and drinking after 1 hour and are fully recovered within a few days.

A small number of male mice are vasectomised, which are used to mate with foster females. Vasectomy is conducted on anaesthetised males by making a small scrotal incision and cutting the thin tube carrying the sperm. The incision is closed and the mice recover from the anaesthetic within 60 minutes or so. A week or two later the wound will be healed and the mice can be used for mating with females.

Transgenic mice in this project will be bred and weaned. Where the line is not pure bred a small piece of tissue from the ear is taken for genotyping the mice to identify the genetic alterations they carry.

Transgenic mice are used for vaccinations also known as immunisations. Vaccination involves injecting a purified foreign substance into the mice, which is known as an "antigen". The vaccinated mice develop antibodies against the antigen and a types of cell known as a T cell begin to divide too.

Immunisation is usually conducted with a very fine needle and a small volume as this is the least painful method. To get a good immune response usually we conduct several vaccinations over a few months, similar to the "MMR" vaccinations we give our children. To check if the vaccination is working a small drop of blood is collected during the immunisation which we check in the laboratory to see if we can find antibodies that recognize the target. In the final stage, the mice are killed and the blood, spleen, bone marrow and lymph nodes collected for analysis.

In some cases instead of purifying a foreign substance from a virus or bacteria for immunisation, it is more efficient to take the gene and put this in the mouse. This is like gene-therapy in that we inject DNA encoding the foreign substance into a blood vessel which then ends up in the liver where it

expresses the foreign substance for several weeks, resulting in a strong immune response. This is similar to the famous Covid 19 vaccine trial from Oxford, in which the DNA that makes a Covid 19 protein is used to vaccinate human subjects.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of the animals in this project should not experience any adverse impact as they are used for mild procedures like breeding and vaccination.

The procedures used for immunisation involve needle pricks for vaccination and to collect small amounts of blood for analysis. Other procedures used for immunisation may be involve injection into a vessel which may involve anaesthesia. In either situation the mice are subdued and lethargic for an hour or two but then recover fully as the affects of the injection and/or anaesthesia wear off.

Some of the animals will experience transient pain from the minor surgery required for embryo transfer and vasectomy. Both of these procedures which will be conducted under anaesthesia and managed with drugs to relieve pain.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mouse : Mild severity : Greater than 98%

Mouse : Moderate severity: less than 2%

What will happen to animals at the end of this project?

- Used in other projects
- Killed
- Kept alive

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The immune system is extremely complex. We have two main types of cells that recognize and kill bacteria and virus infected cells known as B cells and T cells.

B cells make trillions of different antibodies which are released and circulate in the blood. When they find an infected cell they attract other cells to the site of infection to begin an immune response to kill the

virus or bacteria. Each B cell makes a different antibody, but if it encounters a foreign substance on the surface of a virus or bacteria (known as a foreign-antigen) and it is able to bind to it, the antibody produced evolves so that its binds more and more tightly. By the end of an infection the antibodies our B cells produce bind very tightly to their target and are therefore much more effective at killing it.

T cells roam around the body looking for infected cells which they recognise through a unique structure on their surface known as the T cell receptor - which has some similarities to an antibody. Like B cells, every T cell has a different receptor and we have trillions of different T cells receptors too. T cell receptors recognize and bind to foreign antigens, but only do after these have been chopped up into much smaller pieces and presented on the surface of the infected cell. When a T cell encounters an infected cell it starts to divide, it releases chemicals to attract many other types immune cells - such as B cells - and they can also directly kill the infected cell.

The immune response is highly complex. It matures over many months. It involves the interaction of many different cell types and migration of cells to a variety of sites in the body. The interplay between the different cell types simply can't be emulated in the laboratory. This project uses immunisation with foreign antigens to explore the maturation of the immune response, it is not possible to use experimental vaccines in humans.

Which non-animal alternatives did you consider for use in this project?

Some artificial methods using bacteria can be used to discover antibodies in the laboratory, the most well known of which is a technology known as phage display. In this technology viruses that infect bacteria are modified to carry antibody fragments. Large collections of these, known as "phage libraries" can be generated using the genes from human antibody producing cells. Similar technologies have been developed using yeast.

Phage and yeast libraries can be examined to find individual members which bind to a drug target but the antibodies in these libraries are **immature**. They don't bind to their target strongly and they can lack specificity because they bind to other targets, which would cause side-effects.

Antibodies isolated from immunised humanised mice are different - they are **mature**. The difference comes about because the mouse derived antibodies have been induced to evolve into a mature form by immunization. **Mature** antibodies make much better drugs because they bind much more tightly (1000-times or more) to their target - such antibodies can be developed as drugs without further modification.

Artificial methods can also be used to make libraries of T cell receptors from human T cells. However, these libraries will not have T cell receptors that bind to human targets as these are normally removed from the body when a T cell matures otherwise we would all suffer from autoimmunity. However, human T cell receptor genes that bind to human targets can be found in mice with human T cell receptors.

Although these artificial methods provide some options, they can only be experimentally used in isolation. As summarised (and simplified) above, the immune system matures a response, selects and matures ideal antibodies and unique T cells though a highly complex series of interactions involving many different cell types that naturally evolve in an animal over many months. Simply, there are non-animal alternatives that can adequately emulate this highly complex process.

Why were they not suitable?

Artificial display systems can be used to discover antibodies, but in practical applications, these methods have several severe limitations which restricts their usefulness. They are:

- They can bind to several targets, rather than just one.

- They bind weakly to their targets.

- It is difficult to make large quantities and concentrate them.

- Its very hard to find antibodies or T cell receptors which bind human targets.

These issues have to be resolved in the laboratory. This is a slow, error prone, unpredictable and often fruitless process. As a result, despite huge effort only 5% of almost 100 approved antibodies for human clinical use have been derived from phage libraries. Similar issues have been encountered using phage-based methods to turn human T cell receptors (TCRs) into drugs. Powerful and uncontrollable off-target toxicities and human fatalities have resulted.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers of animals estimated to be used for this project are based on 35 years of experience in generating mice with altered genes. They are also consistent with the usage in the existing project license.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

More than 90% of the animals used in this project will be used for establishing and breeding complex combinations genetic alterations.

- Where possible we establish mice which breed true. In these mice both copies of each genetic alteration will be the same.

- As our genetic alterations are made step-wise, we try to completely finishing one before crossing them together.

- We test the function of each genetic alteration before breeding them together.

- The construction of large humanized alleles requires many cycles of genetic engineering. We use embryonic stem (ES) cells for this purpose. We constantly select for ES cell clones that perform well, thereby reducing mice used for donor embryos and recipients.

The other use of mice in this project is for the immunisations required to deliver the core scientific objectives. To reduce mouse numbers we:

- Use standard operating procedures for immunisations and tissue harvesting, ensuring that we get the maximum amount of useful biological materials from each mouse.

- We consult widely to identify and where appropriate integrate improvements in methods to maximise the recovery of data from every mouse

- We have invested in technologies which enables us the sequence the antibody and T cell receptor genes in single cells. By using this technology we get much more information from every immunized mouse. Some years ago we would recover a handful of useful antibodies from each mouse. Today we are able to recover 100-200, which means the mouse numbers required are much lower than they were previously.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

There are four measures we take to reduce mouse numbers:

- We use colony management software to keep track of mice in the animal room, set up just the right number of breeding pairs to produce the mice we need. The data base will record pedigrees and procedures - keeping an electronic health record.

- We conduct pilot immunisations with antigens we have not used before to assess their performance before initiating larger experiments or not conducting them at all in cases where the pilot does not provide a good basis for continuing.

- We stop breeding and preserve mouse lines in cryogenic storage if they are no longer required

- We use contemporary methods to make genetic mutants which avoids numerous cycles of mouse breeding.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice with human antigen receptor loci

The genetic alterations that we have introduced into our mice are mostly mouse/human gene replacements. The mouse genes are exchanged for the human ones which are very similar. Although the resultant mice have a human immune system it is fully functional and consequently the mice are totally healthy. They do not experience any immunological deficiencies.

We also genetically remove the genes for drug targets from the genomes of these humanized mice. This improves the immune response against the drug target. To minimize any unexpected consequence of this type of genetic modification, we carefully check the literature and databases and will not proceed if the mice are not expected to develop normally.

Immunisation Methods

The immunisation methods we use are highly refined and are widely used. We use standard operating procedures and state-of-the-art downstream methods to get the most out of every immunized mouse.

A significant amount of work is performed in the laboratory to maximize the chance that an antigen is able to elicit a good and meaningful immune response. This includes ensuring that the antigen is pure, free of toxins and has not been damaged during its preparation - for example it is has not been degraded or aggregated together. A significant amount of animal use from repeat immunisations is avoided by rigorous application of these principles.

Similarly, carefully preparing the antigen so that it is free of toxic or inflammatory agents avoids unnecessary suffering and focuses the mouse's immune system on the relevant target.

Why can't you use animals that are less sentient?

The immune systems requires several months to develop a robust immune response against a target. The immune system is not mature at an earlier stage (immature life stage).

Lower organisms can't be genetically engineered to the extent possible with mice, the mechanisms of an immune response are different and the structure of the antigen receptors are also different.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

I don't anticipate many refinements to transgenic methods as these are already very standardised and efficient, though where these are reported we will test and implement them.

Where possible we will employ highly experienced animal care staff who are familiar with the specific strains, experimental activities and careful handling of the mice. Familiarity with the balance between welfare and experimental needs, observing recording and reporting expected and unexpected outcomes at the cage-side with score sheets is another essential aspect of minimizing welfare costs. An important balance needs to be struck between to much disturbance of the mice while allowing time pre- and post-procedures for acclimatisation and recovery.

Where possible the mice will not be disturbed unless required for routine husbandry, daily checks or experimental purposes. Environmental enrichments like tubes and nests will be provided.

Where possible mice will be acclimatised, for instance when moved between facilities or animal rooms before any experimental procedures are conducted.

We consult existing literature to ensure we use the latest refinements in experiments. Work will be carried out in state-of-the art facilities by highly trained technicians and scientists, all of whom are dedicated to the highest standards of animal welfare.

The scientists and technicians work closely with the trained and highly experienced personnel in the facility and the veterinary surgeon to ensure that animals experience minimal adverse effects.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I use guidance from the following sources:

- The National Centre for the Replacement Refinement and Reduction of animals in Research (NC3Rs) https://www.nc3rs.org.uk

- The International Mouse Phenotyping Consortium (IMPC) https://www.mousephenotype.org.

- The International Society of Transgenic Technologies (ISTT).

- Some of my team members are members of The Laboratory Animal Science Association (LASA) and attend an annual conference where information on best practice is often exchanged

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

I periodically check website for The National Centre for the Replacement Refinement and Reduction of animals in Research (NC3Rs) https://www.nc3rs.org.uk which has many excellent standard operating procedures and videos.

I'm also a member of an international society where there is frequent technical "chatter" regarding technical advances in the field observations and experience implementing these.

Implementation of a technical variation will usually be conducted with a pilot experiment to gain confidence in the actual method and its reported advantages, ideally with suitable controls. Once this has been assessed and shown to be an improvement then this will be introduced in the standard operating procedure and then implemented as a routine.