Home Office

NON-TECHNICAL SUMMARY

Understanding immune responses to complex vaccines

Project duration

4 years 0 months

Project purpose

- (a) Basic research

Key words

vaccine, gonorrhoea, outer membrane vesicle, antigen, parasite

Animal types	Life stages
Mice	Adult

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 study aims to understand immune responses to complex vaccines, which consist of many different components. The central scientific question is how these combine to induce an immune response which protects against disease in a vaccinated individual.

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?

Bacteria and parasites are responsible for many different infections which afflict both humans and animals. Of these, there are still many infectious diseases where there is no licenced vaccine available, or any such vaccine is of limited efficacy. Some vaccines contain just one component, such as a single protein. Others contain multiple components because they have been derived from, for example, a living bacterium. The general aim of the project is to understand how different components within these types of complex vaccines contribute to an immune response. In principle, if we can improve our understanding of these responses, we can use that knowledge to design more effective vaccines.

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

Outputs from the project will be in the form of publications and presentations at conferences. Specifically, our academic output will examine how vaccines which are made up of multiple molecular components are able to elicit an immune response. An example would be a bacterial extract which is made up of many different proteins and other molecules. We propose to investigate how these different components combine to induce antibodies against many different proteins, and the relationships between them. As another form of output, the data will be shared with the scientific community and offers the potential for comparison with similar data collected from human clinical trials.

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

The primary beneficiaries will be researchers working in the development of vaccines against infections caused by bacteria and parasites. There will also be translational benefit for the pharmaceutical industry- basic research on the immunological mechanisms underpinning vaccines is useful in helping to guide commercial vaccine development which might contribute to combating antibiotic resistance, for example.

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

Our primary output will be publication in learned academic journals. We will combine this with presentation at scientific meetings and conferences. We will make our data available to other researchers on publication- this will be either through dedicated online databases, or as supplementary material included as part of the research paper. We publish papers on an Open Access basis- they are

accessible to everyone, without the need to go through a paywall. The same is the case for the data repositories- the data will be freely available to download.

Species and numbers of animals expected to be used

- Mice: 330

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 species proposed for these *in vivo* studies of immunogenicity is the mouse. Mice are the lowest sensate mammal with a highly evolved immune system that closely resembles our own and are a well-established model for studying vaccine immunogenicity. Because their immune responses are well characterised, we are able to make use of technologies which have been developed by others. A good example is the use of transgenic mice- animals where the genome has been engineered in a specific way to provide an immunological 'marker' which we can use to study how this class of vaccines work. We will use younger (adolescent) mice because they are, from an immunological point of view, naïve- they therefore give us little background in our assays, which would arise from immunological memory.

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

Typically, an animal will be subject to administration of vaccine components by injection. The animal will experience brief and minor discomfort arising from each injection; such administrations will be at least 4 days apart, generally more than a week. No more than 3 doses of any vaccine will be given to any mouse. In the final procedure, the animals will be anaesthetised to collect blood.

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

From our experience, we expect adverse effects to inoculation of vaccine components to be minimal. We pre-screen our vaccine formulations to ensure that they are unlikely to provoke an inflammatory response. In addition, we carry out pilot studies with any new vaccine component or composition, to minimise adverse effects. Some animals may experience localised swelling or reaction at the point of injection, which generally fades after 2-3 days (which we would classify as a moderate effect). Even in these cases, we would not expect any discernible effect on animal behaviour.

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)?

mild (most); moderate (some)

75% mild, 25% moderate

What will happen to animals used in this project?

- Killed

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?

Prediction of immune responses to vaccination in animals is extremely difficult. One reason is that an immune response is dependent on the coordination of responses from multiple different types of immune cells located in different tissues in the body. Immunogenicity- the ability to provoke an immune response- is dependent on multiple factors, including the nature of the molecular species in the vaccine. At present, our understanding of how these various factors operate to produce an immune response is poor. Sadly, we are unable to reproduce these effects reliably using in vitro or in silico (computational) models. One of the ultimate aims of this research programme is to improve this predictability through better understanding and quantitative measurement, which will ultimately help to replace the use of animals. In parallel, we are working with data from human subjects to develop models which would reduce the need for animal experimentation.

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

We have incorporated non-animal alternatives into the experimental programme to ensure that adverse impact of any vaccine tested on the animals used is minimised. This includes the use of cells cultured in the laboratory, which can be used for some measurements. As part of the study, computational methods are also used to select the most promising protein antigens and eliminate any which are predicted to cause adverse reactions. Our vaccine samples are also tested for any contamination which might cause an adverse inflammatory response.

Why were they not suitable?

There is no single non-animal alternative which can accurately reproduce the complex immune responses to a vaccine in an animal. This is because the complexity of the responses requires the interaction of too many cells and components in a way which, currently, cannot be reproduced outside a live animal. The alternatives are valuable in refining the vaccine compositions, but they only test very limited aspects of the immunogenic response induced by each sample. An immunogenic response is dependent on many different factors- the nature of the protein(s) used, the dose, the manner and timing of administration and others. It is currently not possible to predict the amplitude and type of immunogenic response which a vaccine will induce but it is vital to have this information before moving to human trials.

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 estimated are based on refinements that we have introduced under the previous licence, which allowed us to reduce the number of animals needed through careful controlling of experimental variables and appropriate experimental design. We typically now use mice in groups of 5, which is sufficient for us to be able to obtain statistically valid measurements of the stimulation of antibody responses when comparing vaccinated animals with controls, who receive an inoculation without the vaccine components. Each experiment can have 5 or 6 groups, which translates into 25 or 30 mice in each experiment, based on current numbers. The total number of experiments has been calculated to allow us to explore the variety of samples required and conduct sufficient repeats to ensure that our findings are robust and reproducible.

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

We have made use of previous experimental data to refine experimental design in an iterative manner, reducing animal number to a minimum needed to obtain a clear answer to the experimental question posed. Our experimental design compares responses between groups of animals who are administered the same vaccine and makes pairwise comparisons to derive statistically valid conclusions about the effects of particular parameters (eg the effect of certain antigens, dosage level, inclusion of adjuvant). Using these estimates, we have devised an experimental strategy which allows us to derive the maximum amount of necessary information for the minimum number of animals tested. This generally takes the form of an investigation into the strength and direction of immune response to a particular vaccine.

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

We have used computational methods and studies on isolated cells to screen our vaccines before administering them to animals. Computational methods allow us to identify which parts of each specific protein antigen are most likely to elicit an immunogenic response; such methods are not infallible, but they provide a useful basis from which to refine our vaccine composition. In addition, we make optimal use of the tissue from each animal, making multiple measurements from each individual. For example, we can break down the antibody response to a complex vaccine into its components, and thus understand in more detail how antibody response patterns may align with protection elicited by a vaccine. We incorporate animals used from pilot studies into experimental groups, thus reducing the

total number of animals used. Finally, we will use an iterative approach, through cycles of experiments, to identify the most important parameters and optimise them as efficiently as possible.

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.

We have selected the mouse as the model organism for these studies, for the following reasons:

1. The mouse is the lowest sensate organism that can practicably be used for vaccination studies which will be translatable to humans.

2. The mouse immune system is well studied: immune assays (such as cell fractionation) are therefore well established, and suitable reagents for them are readily obtainable.

3. Mouse genetics is well understood: we can make use of transgenic animals which have been made by other investigators.

Our experimental plan reduces vaccination to the simplest and least obtrusive method possible- up to three inoculations per animal, delivered over several weeks. Animals will be handled in such a manner as to minimise stress during inoculation. As detailed above, we will make extensive efforts to ensure that the administered vaccine material is not harmful or likely to trigger adverse responses. Safety and the avoidance of unwanted side-effects is an extremely important aspect of vaccine design. All samples will be tested on a smaller group of animals first, to verify no unanticipated adverse reactions. All animals will be monitored in the 48-hour period after inoculation for weight loss and any signs of distress or discomfort.

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

We require animals with an immune system which is as closely similar to humans as possible. Less sentient species have immune systems which are too different from humans, so the results provide a poorer indication of the likely responses in a clinical trial. Mice are a well-established model for vaccination studies: our findings will therefore be easy to relate to those by other investigators who have carried out related, but different, experiments. Non-mammalian animals differ too much in their immune function from humans to be useful for vaccine studies. The use of embryos or very young animals is also not feasible because immune systems need to develop to a mature stage before they can be used as suitable models for the human immune response. Terminally anaesthetised animals cannot be used because it takes weeks for immune responses to develop and it is not feasible to keep animals under anaesthetic for that long.

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

Pilot studies will be carried out whenever a novel vaccine composition is introduced. All animals will be monitored after vaccination for reaction at the site of injection 6 and 24 hours after inoculation. In addition, animals will also be monitored in the 48-hour period after inoculation for signs of distress and altered behaviour (eg change in weight, hunched posture, reduced activity levels, altered social interaction). Observations of adverse reactions will be used to inform the design of future vaccine formulations- for example, we may reduce the level or number of doses where a particular vaccine component is the cause of the reaction.

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

We will make use of:

1. The PREPARE guidelines (https://norecopa.no/prepare) provide resources on study preparation, dialogue, education, communication and quality control of sample.

2. The ARRIVE guidelines (https://arriveguidelines.org/about) which covers similar topics to PREPARE, but extended to manuscript preparation and review. Many scientific journals now require completion of an ARRIVE checklist before publication.

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

We will use the newsletter and website of the NC3Rs, which is a valuable hub for dissemination of good practice in animal experimentation, with particular attention to innovations in the study and development of vaccines. We will also incorporate good practice from other specialists in the area: a good example is working with national bodies involved in replacing, reducing and refining animal use for vaccine testing. Staff engaged in the project will, in addition to mandatory training, be encouraged to engage with relevant webinars from NC3Rs and elsewhere which provide up-to-date advice and information on best practice.