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NON-TECHNICAL SUMMARY

Use of genetically modified biological assemblies to generate improved vaccines

Project duration

4 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

Key words

Vaccine, Infection, Virus, Immunogenicity

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?

This project will apply genetic modification to proteins which spontaneously assemble to form cage-like structures. I aim to demonstrate that these assemblies can be used as ‘scaffolds’ to form the basis for development of novel candidate vaccines against different infectious diseases, including Covid-19, whipworm and gonorrhoea.

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?

There are many infectious diseases for which effective vaccines have not been developed, or provide limited protection in vulnerable people (eg the elderly). Vaccines are a highly effective way of protecting the public and have been instrumental in reducing or eliminating many infectious diseases. New technologies offer the potential for the development of more effective vaccines against a wide range of different diseases, but they require testing in animals to ensure their safety and efficacy before their use in human trials.

This proposal relates to a specific technology which we have developed relating to the use of protein assemblies: this general term refers to the use of protein subunits which assemble into spherical structures. Such assemblies can be used to display specific vaccine antigens: antigens are molecules which are able to invoke an immune response. Vaccines incorporate antigens from infectious organisms and use these to stimulate a protective response, often by stimulating the production of antibodies against them. Protein assemblies are particularly effective at doing this: they can therefore be thought of as ‘scaffolds’, in the sense that they provide a molecular superstructure to which particular antigens can be bound. It is well established that virus-like particles can be used as a type of scaffold- these are virus proteins which assemble into a viral particle or shell, but are not capable of causing disease because they lack most of the components necessary for infection. Such assemblies are known to have useful immunological properties which make them particularly effective as vaccines. Our technology allows the coupling of multiple antigens to a molecular ‘scaffold’ or support, in such a way that vaccines can be formulated easily with multiple components. This is a generic method, in principle applicable to the development of a vaccine against any infectious disease. We expect it to be particularly valuable for vaccines which require multiple components, or the immune response needs to be modulated in a specific manner. We anticipate that it may be able to overcome challenges which have limited vaccine development against specific diseases, including poor immunogenicity or an inappropriate immune response.

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

I anticipate that I will be able to demonstrate appropriate immunogenic responses to antigens derived from several important human pathogens, including the Covid-19 virus, the bacterium *Neisseria gonorrhoeae* (responsible for gonorrhea) and the whipworm parasite.

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

The recent Covid-19 pandemic has highlighted the importance of developing new vaccine technologies, particularly to protect against diseases for which there are no effective treatments, or where there is escalating resistance (eg to antibiotics). This project aims to validate a generalised approach to this problem which could be applied, in principle, to any infectious disease. The impact could be wide-ranging but will be after the conclusion of the project: vaccine development is generally a slow process and it typically takes many years for a new product to reach the market. It is important to note, however, that vaccines have saved the lives of millions of people and animals worldwide. We urgently need new approaches to address those diseases which, for a variety of reasons, have been resistant to vaccine development up to this point. It is only through further research and innovation that we will be able to overcome these challenges.

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

The results will be disseminated primarily through publication in reputable peer-reviewed scientific journals, to ensure that it is subject to the scrutiny of the scientific community. The work will be presented at international conferences, which provides an opportunity for informal feedback and discussion. I will also seek to develop the work commercially: if appropriate, I will seek patent protection for any inventions which emerge from the project.

Species and numbers of animals expected to be used

- Mice: 650

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.

Development of new vaccines requires measurement of their ability to generate protective immune responses. The principle of a vaccine is that it attempts to reproduce the protective response of the immune system to an infectious agent- a virus, bacterium or parasite. Orchestration of the immune system is complex, involving many different types of specialised cells distributed throughout the body. Although aspects of the immune response can be modelled *in vitro* to a limited extent, it is not currently

possible to reproduce it in its entirety without studying responses in the whole animal. Mice are commonly used in vaccine studies, making our results easier to compare with those obtained by others. Adult animals are most suitable for these experiments.

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

Each mouse will be inoculated up to 3 times with a small quantity of an experimental vaccine. Each dose will be separated by about 10 days; at the end of the experiment, each animal will be humanely killed before collection of blood and body tissues for analysis. Animals will be routinely monitored for any unanticipated adverse effects.

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

The impact of our experimental vaccines is expected to be minimal- they consist of non-infectious protein and are unlikely to cause any harm to the animals. To minimise the risk of this happening still further, our candidate vaccines are tested for toxicity before administration. Vaccines generally consist of low quantities of material and are designed to be safe; adverse reactions are rare.

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)?

It is expected that all the mice will experience only mild discomfort during the inoculation steps. Given the low dose and minimal toxicity of the administered vaccine material, I anticipate that the majority of inoculated animals will experience no or minimal reaction. It is possible that a minority of inoculated animals may experience a reaction; for this reason, the maximum severity expected is designated as moderate.

What will happen to animals at the end of 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?

It is not possible to reproduce the highly complex immune response induced by a vaccine without experimentation on the whole animal. Vaccines induce immune responses which require the coordination and interaction of multiple different cell types: B-cells, T-cells, dendritic cells and others. This complex network of interactions had not been modelled to a level of precision which can substitute

for experimentation on the whole animal. Moreover, it is critical that we verify our vaccines are safe, as well as effective, before testing them in humans.

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

The following non-animal alternatives are already in use as part of the project, to optimise the vaccines before testing on mice. I use computational methods to select the most promising protein fragments to use, and eliminate any which are predicted to cause adverse reactions. I also use dendritic cells to test for vaccine uptake and identify whether any of our candidate vaccines are cytotoxic (can kill cells). Dendritic cells are cells which play an important part in processing antigens and directing the immune response. Our vaccine samples are also tested for any contamination which might cause an adverse inflammatory response.

Why were they not suitable?

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?

Our estimate is based on our previous experience, and published details from others, who have administered similar vaccines to mice in the past. We have modelled the effects which we anticipate and have based our estimate of total numbers on those statistical predictions.

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

I have used statistical modelling, including the NC3R's Experimental Design Assistant, to model the effects which I anticipate for the vaccines I propose to use. 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, I have devised an

experimental strategy which allows us to derive the maximum amount of necessary information for the minimum number of animals tested.

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

As detailed in previous sections, 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. We can 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.

I will use the mouse as a vaccination model. The minimal requirement for such an experiment is the administration of vaccine, in up to three doses, through a suitable route. Each animal will therefore only be subjected to a maximum of three inoculations. Animals will be handled in such a manner as to minimise stress during inoculation. As detailed above, I 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 24 hour period after inoculation for weight loss and any signs of distress or discomfort.

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

The mouse is well established model for vaccine studies. 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. In addition, there is a large body of published evidence which enables a direct comparison of the effects of particular vaccines in mice and humans. By doing our trials in mice, I will be able to compare our data directly with those findings and use it to extrapolate to the likely responses

and safety in human subjects. I cannot use terminally anaesthetised animals 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?

I will conduct the minimum of experimentation necessary to collect the data which I require to exemplify the invention associated with this project. The work will proceed in experimental cycles, several months apart, which will allow me to learn and adjust the parameters of each experiment as I proceed. I will therefore refine critical parameters such as antigen selection, dose level and dosing strategy in an incremental manner, optimising immunogenic responses and eliminating any adverse effects, if observed. Refinement of the procedures, including optimised mouse handling techniques, is therefore an intrinsic part of our experimental approach.

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

In addition to NC3Rs, technical advice and guidance is available to licence holders from the government (https://www.gov.uk/guidance/animal-research-technical-advice). Another valuable source of information is from regulatory bodies; for example, the Federal Drug Administration (FDA) has recently produced guidance for developers of COVID-19 vaccines, with specific advice on animal models (https://www.fda.gov/media/139638/download). Finally, I will continually survey the academic, peer-reviewed literature for publications which describe animal vaccine experiments which are similar to our own, to avoid duplication and allow us to refine our protocol further.

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

The NC3Rs produces a newsletter and is a valuable hub for dissemination of good practice in animal experimentation. Staff engaged in the project will, in addition to mandatory training, be encouraged to engage with webinars from NC3Rs and elsewhere which provide up-to-date advice and information on best practice.