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NON-TECHNICAL SUMMARY

Intrinsic & extrinsic effects on B cell differentiation

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

B cell, Immune response, autoimmune response, ageing

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

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?

B cells are the cells that produce antibodies after vaccination. We plan to study B cell activating factors - signals that are transmitted through surface receptors on B cells or are delivered by cells that interact with B cells.

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?

Our bodies can defend themselves from infections by producing antibodies. Antibodies are proteins that recognise and bind specifically to bacteria or viruses (pathogens). This then leads to the destruction of these pathogens by the rest of the immune system. Antibodies form after infection by a pathogen and can give life long protection from that specific pathogen. The result will be the generation of specific antibodies and long term immunity. Antibodies are produced by B cells. The purpose of this project is to understand how B cells are activated by pathogens, how this makes B cells develop antibodies that are highly specific for a pathogen, and how the B cells transform into cells that make huge amount of antibodies, or how B cells transform into memory B cells that survive for a very long time protecting us from illness for many years.

The Covid pandemic was a reminder how important vaccination and the rapid design and generation of vaccines specific to new threats is. Vaccination is a way of mimicking infection by a pathogen without the danger of developing an illness from that pathogen. Vaccination leads to the development of antibodies in exactly the same way to what happens during natural infection by pathogens. Because the fundamental processes how B cells develop and how they interact with other immune cells are not well understood, it is still difficult to design and generate vaccines in a targeted way. Understanding these processes better may lead to more intelligent ways to generate vaccines that induce protective B cell responses.

During ageing our body reacts far less efficiently to vaccination or produces antibodies in response to infection. Understanding the defects that inhibit responses in the aged will help create treatments that may enhance the aged antibody response.

Antibodies not only protect, but they can also generate illness. Some people develop autoimmune disease where B cells start making antibodies to structures in our own bodies. How B cells are triggered to make antibodies to ourselves is another part of this project. Understanding this may lead to new ways how to treat or prevent autoimmune diseases.

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

After vaccination, B cells can fine tune the specificity of antibodies they generate towards pathogens. The project will create information on the fundamentals how this process works, how cells interact to regulate the process, and what kind of signals are exchanged. We will create scientific publications about our results. Further, we will publish and discuss our data with others working in the field on

scientific conferences. This will benefit others working on understanding vaccination or the antibody defence to pathogens to better understand these processes.

B cells themselves can also cause disease by inappropriately getting activated to react to our own bodies. This is called autoimmunity. Understanding the factors that activate B cells to vaccines will also help understanding inappropriate activation of B cells triggering autoimmunity. By the end of this project we plan to have published scientific data on this. This work will facilitate the work of others studying autoimmunity, how to prevent autoimmunity being induced, or how to cure autoimmunity.

In the longer term this work should lead to knowledge how to better induce antibodies in animals, for example during the generation of new antibodies that can be used as human drugs. It should lead to better ways to generate human vaccines to pathogens or enhance antibody responses to vaccination or infection in aged people. Understanding autoimmune processes should lead to the development of drugs that can prevent autoimmunity or cure autoimmunity.

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

Fundamental research

Scientists studying B cells, antibody responses, vaccination, and the immune response to pathogens will directly benefit from this work in the short term.

Biotechnology

Scientists developing vaccines to pathogens, monoclonal antibody drugs targeting a huge range of diseases, or drugs directly targeting autoimmune disease should benefit from information that will enable their work in the medium term.

Patients

In the long term, patients may benefit from improved vaccines or vaccination schedules, drugs enhancing antibody responses to infection or in aged patients, and drugs targeting autoimmunity.

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

The output of this project will be presented and discussed at national and international conferences. Outputs will be published in high profile open access journals. The public and peers will be informed about these by press releases and social media. Manuscripts for publication will be prepared according to ARRIVE guidelines.

We collaborate with clinicians who work on infection, aged patients, or patients with autoimmune disease. We collaborate with industry who develop genetically engineered mice to facilitate the rapid generation of human antibody drugs.

Species and numbers of animals expected to be used

- Mice: 35,720

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.

We are using adult mice, because they are the only well studied mammal that has an immune system that is sufficiently similar to the human immune system. Lower vertebrates than mammals lack many of the features of the immune system we share with mice and other mammals. Only in mice experimental methods have been developed that will allow us to undertake our work.

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

Typically, mice will experience mild, transient pain and no lasting harm from immunisation. Immunisation will be done by injection using standard routes or injection into the foot. Foot immunisation will be done under anaesthesia and may lead to short-termed mild swelling that will not affect normal behaviour. Mice may be reimmunised or injected with immunomodulatory substances. No animal will be injected more than four times. Blood samples will be taken at the beginning of the experiments, before and after immunisation. Most animals will be killed within 10 weeks of immunisation. The test substances will already have been tested to ensure that the dosing regimen does not cause toxicity. The final procedures will be undertaken under non-recovery anaesthesia where the animals will only be aware of the anaesthetic being administered and may experience mild distress and no pain.

When we use infectious agents that replicate, we will prioritise the use of attenuated bacterial or viral strains, live vaccines, or expose to doses of infectious agent from which we expect the animals to recover after experiencing moderate severity. This may result in some discomfort similar in duration and severity similar to that of a vaccination or infection, but is rarely found to lead to severe outcomes.

Some animals will be treated to induce autoimmune responses. In a model for arthritis, mice will be injected with agents that induce antibodies that can lead to arthritis. In a model for Sjogren’s disease, fluid containing a virus will be delivered through the salivary gland duct under anaesthesia. As these experiments are performed to study the induction of antibody responses, most animals will only be maintained until the autoimmune antibody response has formed and most experiments will be terminated before autoimmune disease can develop.

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

For most of the mice, including immunodeficient strains, we do not expect any impacts or adverse effects in our AAALAC accredited specific pathogen-free (SPF) animal care facility.

Embryo transfer and vasectomy are surgical procedures with short term post-surgical pain. Post-surgical pain will be controlled by analgesia and any animal not fully recovered (eating, drinking, return to normal behaviour) within 24 hours will be euthanised.

The adverse effects of immunisation/infection or immunomodulation include systemic or specific tissue inflammation which will be transient, lasting for a few days. In the case of influenza virus there will be substantial weight loss which is restored within two weeks. Appetite stimulants are fed in mash in addition to normal diet during the course of the experiment to try and ameliorate the percentage of weight loss.

Some viruses will induce chronic infections with adverse effects such as weight loss and while this may be moderate in C57BL/6 other inbred strains manifest enhanced vascular permeability, lung immunopathology and animals will be closely monitored according to the humane endpoints detailed.

Foot immunisation, done under anaesthetic will often lead to foot swelling that can last a week, but does not affect normal behaviour.

Although ageing is a major risk factor for adverse effects, we know that the vast majority of our aged mice remain healthy throughout the duration of their lifetime. There is an increased incidence of adverse effects not observed in young wild-type mice including altered coat condition, diarrhoea, eye abnormalities, abdominal distension, movement issues, tremors and seizures. A tiny minority of these develop tumours, but regular checking by our experienced animal technicians ensures these are detected early, and the mouse euthanised immediately. A specific code of practice for caring for aged mice is in place.

All forms of arthritis cause joint stiffness and some degree of disability for the duration of the study (100% incidence). Arthritis will be monitored carefully using a scoring system that monitors and scores behaviour; coat condition; body weight; mobility and weight bearing; grimace and calliper measurements of the injected joints. Measures will be taken to ameliorate these adverse effects eg soft bedding on the cage floor.

Mice will have salivary gland cannulation under the anaesthesia, which may cause stress, and slight swelling of the salivary glands post cannulation. The swelling usually resolves in 48-72 hours.

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)?

The expected severity for most of the mice will be mild or sub threshold

- Total animals used = 35,720
- Mild: 37% (13,070)
- Moderate: 5% (1,750)
- Sub threshold: 59% (20,900)

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?

We need to use the animal model to understand how antibodies are generated in response to vaccination. This process happens in lymphoid tissue such as spleens or lymph nodes. Lymphoid tissues are very complex structures with many different cells types interacting and communicating with each other, and cells migrating through different sub-compartments that provide different environment with different interacting cells and stimuli. These processes are so complex that currently no in vitro system is able to replicate this.

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

1. We are collaborating with physicists that computer model immune responses.

2. In vitro models, where cells interact in test tubes in solutions outside the body are generally not sufficiently complex to recreate the complex sub-compartments in lymph nodes.

3. We are currently working on modeling the interactions happening between immune cells using proteins or stromal cells in the test tube (in vitro) in the lab.

Why were they not suitable?

1. Whilst computer modeling of immune responses is ongoing and improving these do not yet begin to replicate the complicated conditions and variables that exist in the immune system. Our use of computer models helps to confirm some of our data and provide testable hypotheses but is limited in scope and output to small aspects of the B cell response in vivo. While reducing animal experiments, computer modeling typically creates new hypotheses that have to be tested in the intact organism.

2. In vitro models can be useful to replicate and study processes during B cell stimulation in the lab. They are used in our laboratory when appropriate, but do not replicate the complexities of the environment in which immune responses occur.

3. Similar to computer modeling or modeling of immune responses using proteins or stromal cells in the test tube (in vitro) can reduce the number of experiments, but also creates new hypotheses that have to be tested in the living organism.

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?

We have estimated the number of animals we will use based on our previous studies using these protocols. The numbers of mice required for the generation and rederivations of genetically altered mice are based on extensive experience of staff who regularly perform these protocols.

The use of colony management software and knowledge of the breeding performance of individual strains has enabled us to predict the numbers of mice of the correct genotype that we will produce from breeding, and the numbers of aged mice that we will need. Sample sizes for our experiments are estimated from past experiments, with power calculations done with support from the local statistician at our Institute using exemplary data from earlier studies

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

Advice from local professional statisticians has been sought to evaluate proposed experiments for statistical validity and in the generation of power calculations. Numbers of animals necessary were calculated using our own historical data to estimated the expected variability of our experiments and to calculate the minimal number of animals necessary to generate significant results.

Further, we are continuously refining our analytical methods and use the NC3R's Experimental Design Assistant to ensure we are considering all relevant aspects of design, in order to reduce variability, allowing further reduction in animal numbers. All relevant tissues where possible will be frozen and stored as input and controls for downstream experiments. Shared use of these will be offered for shared use by other groups working on related questions.

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

We will minimise use of animals by keeping colony sizes as small as possible. This may lead to experiments having to be split into two independent repeat experiments to generate sufficient power. New substances used on animals will be tested first in small pilot studies. Computer modeling may be used to predict experimental conditions that will show the largest effect sizes. At the end of the experiment, we will harvest the maximal possible number of tissues. Tissues not immediately analysed will be archived in frozen state and will be made available to other researchers working on similar questions.

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.

Some animals will be allowed to age to study the immune response in the ageing organism.

In order to induce gene expression in animals or deplete specific cells some animals will have receive substances via gavage (force-feeding), injection, or through food. Oral gavage or injection can be necessary to induce a rapid onset of gene expression. Use of flexible plastic gavage tubes over metal fixed cannula will reduce risk of trauma and inflammation. This will allow us to study processes that happen within short time periods of a few hours.

We will have to induce immune responses to study the response to vaccination. Animals will be vaccinated using methods similar to human vaccination, e.g. injection of substances under the skin, intramuscular injection or by intraperitoneal or intravenous injection.

Some animals will have to be vaccinated into the foot, as this induces a strong response in local lymph nodes. This will be done under short term anaesthesia; however, the animals will suffer from temporary swollen foot for several days. This has not led to changes in normal behaviour in the past indicating that there is no major discomfort. If animals show signs of significant foot swelling or inflammation, they will be treated with analgesic agents.

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

Non-mammalian animals are limited in their use because they either do not possess B lymphocytes or their differentiation in response to stimulation is too removed from the human immune system to provide relevant results. Embryos are unsuitable as their immune system is not mature and does not respond to antigenic stimulation in the way mature animals do.

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

Ageing animals will be carefully monitored by trained staff to work with ageing animals. Group sizes in ageing experiments will be increased to accommodate for loss of animals and to avoid single housing due to animal losses due to old age. Animals will be monitored for adverse effects such as changes in weight, dermatitis, piloerection, paleness, changes in mobility, lumps, eye defects, abnormal respiration or stools. If these are observed animals will be treated accordingly, and animals with that may develop severe effects will be killed humanely.

If gene induction or deletion is done for a new gene animals will be monitored closely in the days after induction. Mice will be weighed daily to detect weigh loss. If any mice have reduced activity, ruffled coat or hunched appearance they will be warmed and given glucose-saline (subcutaneous injection) to reduce heat loss and dehydration which may be a contributor. Flexible gavage tubes dipped in sucrose will be used to minimise damage to the oesophagus. Refined mouse handling technique and technical expertise will minimise any discomfort. Time and route of induction will be optimised in preliminary

experiments for efficient induction or deletion of transgenes using the least adverse route of administration.

For all new models, new methods and new antigens we will consult with expert staff at our animal facility. Some methods, e.g. animals that are receiving new antigens or immunisation protocols, will be carefully monitored during the protocol and humane end points will be used if necessary in consultation with expert staff at the animal facility.

Immunisation will be done via injection of antigen. These vaccinations should only have transient effects and animals should return to normal behaviour within two hours. If new antigens will be tested, this will be discussed with expert staff at the animal unit. New antigens will be tested on small groups of animals first.

Some animals are immunised in the foot under short term anaesthesia. Foot immunisation may lead to foot swelling due to inflammation. This should not be strong enough to lead to behavioural changes. Mice that do show excessive inflammation or lameness will be treated with analgesics. Refined mouse handling technique and technical expertise will minimise any discomfort. Mice are not re-injected until fully recovered from previous injection and never at a frequently that causes them to display anything other than transient pain to discomfort. Foot immunisation has been refined by injecting substances under the plantar surface of the foot, away from the weight bearing walking pads. Further, injection of non-immunogenic substances will be done by injection into the hock above the foot.

LASA guidelines will be followed regarding volume of substances to be administered.

Pathogens used are weakened or killed versions that should be non-pathogenic within the time-scale of the experiment. When pathogens are used, animals will be monitored appropriately. In the case of bacterial infection mice will be monitored for the first 48 hours following immunisation and also during the third week of infection which is the time of high susceptibility of secondary infection. The dose of bacteria administered is the lowest possible to obtain a response. Expert animal handling staff are aware of the course of the infection and adverse effects are expected to be noticed and dealt with quickly.

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

Experiments will be performed in line with LASA guidelines, and using the NC3Rs Experimental design tool.

Will ensure that all experiments are designed to allow reporting in accordance with the ARRIVE guidelines. Our research will be published according to ARRIVE guidelines, regardless of whether a journal endorses this.

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

We will regularly check information on NC3Rs, have signed up to the NC3R newsletter, will use the regular NIO newsletters containing latest 3rs advances and opportunities, and attend Regional 3Rs

symposia.