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NON-TECHNICAL SUMMARY

B cells in tumour immunity

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

B cell, cancer, vaccine, autoimmunity

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?

Characterising the function of B lymphocytes in immunity to tumours. Developing a new tumour-specific vaccination approach that activates B lymphocytes.

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?

Cancer is a main cause of death of the aged, but also of younger patients. New and cost-efficient therapies are clearly needed. Recent major advances in cancer immunotherapy have shown that the immune system is able to attack and destroy cancerous tumour growth. Still, harnessing the immune system to specifically attack structures on tumour tissues is a major challenge. Tumour tissue is made of the patient's own tissue, i.e. it consists of immunological self-structures (or autoantigens). The problem with inducing immune responses to self-structures is that our immune system is very good at avoiding attacking immunological self in order to avoid autoimmunity. This phenomenon is called immunological tolerance to self.

A now established therapy against cancer is that monoclonal antibody drugs are injected that target human self-antigens expressed by tumours. These antibodies are originally generated in animals. In animals these human antigens are foreign, so self-tolerance is not an issue and antibodies are produced after vaccination. Generating monoclonal antibody drugs, and the production, storage and administration of monoclonal antibody drugs are expensive and laborious, and antibody drugs last in the patients only for a limited time.

We plan to harness vaccination in order to let patients generate their own antibodies against self-structures on the tumour. Advantage of vaccination is that it is a well-established method against pathogens. Vaccines can be generated and administered cheaply and therefore have been used widely for therapy and prophylaxis in underdeveloped parts of the world. While vaccination against self-antigens should be difficult, as immunological tolerance prevents responses against self, we have developed a new vaccine conjugate that should be able to overcome self-tolerance and efficiently induce self-specific and tumour specific responses.

In the current project we plan to develop and test variations of this vaccine. At the same time, we plan to study interactions of B cells – the cells that produce antibodies to tumour structures – with tumour tissues, and the role of immunological self-tolerance in the interactions of B cells with tumour tissue.

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

The project will generate better understanding on how B cells interact with the environment in tumours (tumour cells and immune cells). Outputs will be presentations at scientific conferences, publications in scientific journals, and/or patents.

The second aim of this project is to optimise our vaccine design that is able to induce antibody responses to self-structures expressed on tumour tissues. We plan to generate variants of this vaccine and test their efficiency in pre-clinical animal models. Outputs during this project will be presentations

at scientific conferences, publications in scientific journals, and/or patents. In the long term the project shall lead to the development of new vaccines that will allow preventative or therapeutic vaccination to cancer.

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

In the short term, this project will have broad academic benefit, provide new information on how antibody responses are triggered to self-antigens after vaccination, and new methods how of inducing self-antigen-specific antibody responses. Mouse models of tumour development will provide information of B cells in primary tumours and tumours after vaccination, which will increase our fundamental understanding of B cell activation in different tissue environments, and in response to the tumour or to vaccination.

Further, it will let us test new versions of our cancer vaccine and its prophylactic and therapeutic efficiency against tumour growth in mouse tumour models.

In the medium to long term this project will impact on clinicians and patients: If translated into clinical use, this vaccine has potential benefits for the prevention or treatment of cancer by vaccination. Cancer vaccines have the potential to bring major benefits in treating antigen-expressing tumours, either alone, or as adjuvant or in combination therapies, to improve cancer treatment. This approach also has potential benefit for other disease types where self-antigens might be targeted.

Commercialisation would benefit UK biotech industry in the long term (>10 yr).

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

This interdisciplinary project links groups working in immunology, cancer immunology, tumour biology, and clinical cancer medicine. We will present findings locally through shared meetings, in our animal facility and local institutes which study Cancer and Immunology. This large base of scientists and clinicians interested in immunotherapy of cancer will provide opportunity for interdisciplinary discussion and project support from the local community, maximising the opportunities for new collaborations.

Results of the work will be published in high quality open access journals according to the ARRIVE guidelines and presented at both national and international scientific meetings, and the local institute websites regularly updated. We plan to publish unsuccessful approaches as well.

New approaches may be patented, which will maximise the potential to translate this into successful biotech products that become quickly available at large scale.

Species and numbers of animals expected to be used

- Mice: 1000

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.

Mice will be used in this project as preclinical models, studying the complex interplay of the local immune environment in tumours and lymphocytes, that are activated in lymphoid tissues distant from the tumour. At present, there are no models that replicate the complex interplay of different immune cells and stromal elements in tumours and lymphoid tissues in vitro.

The adult stage will be used as the antibody immune response is only fully developed in the adult. Further, immune tolerance to self-antigens will be studied, and this is only fully developed in the adult organism.

Mice are the most appropriate animal, because as mammals their immune system has a huge degree of homology with the human immune system, their immune system is best studied, and there is the largest number of experimental tools available to manipulate and analyse their immune response (e.g. gene manipulated animal strains, antibodies to detect specific proteins, and gene expression detection reagents).

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

Subcutaneous tumour models will be used in this project.

Tumour cells will be injected once subcutaneously on a single flank site. Tumour sizes will be measured regularly.

Vaccines will be injected prior to or after the tumour implantation via subcutaneous injection.

Blood sampling may be taken during the tumour growth from peripheral blood vessels in accordance with LASA guidelines.

After not more than 6 weeks the animals will be killed by a schedule 1 method.

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

Implantation of tumour cells under the skin may cause adverse effects. The adverse effects are expected to be transient and mice are expected to return to normal behaviour within 2 hours of implantation.

Tumour implantation may be done under short general anaesthesia. Mice will be appropriately monitored during anaesthesia. Mice will return to normal behaviour after waking up from anaesthesia.

Growth of tumour cells under the skin in some mice may cause adverse effects such as distress or pain leading to weight loss. The animals will be closely monitored after the implantation. They will be schedule 1 culled if any adverse effects are observed which exceed moderate severity (including weight loss (maximum of 20%), a body condition (BC) score of <2.5, reduced activity, continuous

hunched posture et al). Subcutaneous tumours will be measured maximum length x maximum breadth in mm. These two measurements will be used to estimate the tumour volume. If this exceeds 1.25 cm^3 then the animal will be killed.

Treatment with vaccine (e.g. immunogens, adjuvants). These may have side effects. The type of immunogens and adjuvants proposed for use in the project generally have few side effects, however we will use previous experience and data in the scientific literature, plus pilot studies to inform our use.

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 level in all animals is moderate. The majority of animals will have a tumour implanted, and the majority of these will be vaccinated. A minority may encounter vaccination only in order to study the immune response to self-antigens.

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?

Aims of this project are to test the role of B cells in tumour immunity, and developing a therapeutical vaccine targeting tumour blood vessel development, inhibition of tumour growth and spread. This is going to develop data to support clinical trial. At present, there are no models that can mimic the complex interactions of the immune system (lymphocytes and lymphoid organs where lymphocytes are primed) with tumours, or the development of an immune response to a vaccine in vitro. Therefore we are dependent on mouse models.

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

1. In silico analysis of interactions of the immune system with tumours and of vaccine responses

2. In vitro B cell activation

3. Analysis of immune responses in human tumour tissue

4. Review of the scientific literature

Why were they not suitable?

1. We have been developing and published in silico methods to analyse immune responses, however, these are only able to test hypotheses that have been developed with data derived from in vivo experiments. They are able to inform about optimal design (e.g. optimal time points or doses) of future in vivo experiments and generate new hypotheses, but these new hypotheses have to be tested again by in vivo experiments.

2. Wherever possible, we do in vitro experiments to study individual steps of B cell activation and differentiation in vitro. In vitro models are useful for variation of simple processes immediate after B cell stimulation, however, do not replicate the immune system and the complexities of immune cells interacting with each other and their environment, i.e. in the tumour or in lymphoid tissues.

3. We are collaborating with local colleagues who do analyse fixed explant tissues from tumour patients. However, this is not sufficient to analyse the complex interactions during the initiation of an immune response, interactions between the tumour and local lymphoid tissues where the response is started, or model the complete anatomical, and cellular interactions.

4. We do continuously review the scientific literature and will adopt our research plans accordingly.

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 experience from our published studies on vaccination of tumour implanted mice about the expected data variation and group sizes necessary that will provide sufficient statistical power. Individual experiments comparing two parameters would need 30 mice.

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

The experiments are based on our earlier published studies. Therefore, we have extensive experience on techniques of studying tumour growth and experimental data variation. Future experiments will be planned and performed in collaboration and under guidance of our coauthors on this study, who have long-term experience in studying tumour growth and vaccination. This will help establishing optimal tumour implantation techniques to reduce experimental variation.

We will reference online tools NC3R's Experimental Design Assistant for experiment design, statistical analysis to predict the numbers of animals used in the study.

For each experiment, we will produce a written protocol. In this the numbers of mice used and the procedure they will undergo will be details, according to the ARRIVE guidelines.

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 apply good colony management to ensure the desired numbers and desired genotypes are generated with minimal wastage. Computer modelling 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 frozen 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.

Mice will be used in all experiments.

Tumour models will be used to study B lymphocyte infiltration in tumours and interaction with other cells.

Mouse tumour cells (e.g. Lewis Lung Carcinoma Cell) will be subcutaneously injected into one single flank site of wild type mice or B cell reporter mice. Comparing to tumours that develop in the organ from which they are derived, subcutaneous implantation of tumours is one of the most refined tumour models because it minimally invasive method to administer cells, and subcutaneous tumours can be more closely monitored, allowing for better HEPs, and less likely to impact on animal welfare than those growing in organs and potentially impacting upon organ function. This will be the model of choice to determine how vascular targeting by vaccination and immune responses affect primary tumour growth.

Vaccination will be used to induce anti-tumour immune responses and test interactions of the immune response with the tumour. Mild adjuvants have been selected for immunisation that avoid significant pain or distress to the animals.

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

Mice are the most appropriate species, because they have a huge degree of homology with the human immune system, and have been used extensively in previous studies meaning there is a wealth of information and established techniques available to us (e.g. gene manipulated strains, antibody and

gene expression detection reagents). Adult mice have to be used because only the adult immune system efficiently reacts to the self-antigens we are planning to target with our vaccination strategy. The experiments run over several days and weeks and therefore cannot be done under anaesthesia.

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

Procedures that may cause acuter pain, e.g. implantation of tumour cells by subcutaneous injection, will be done under short term anaesthesia.

Treatment with vaccine (e.g. immunogens, adjuvant) or modulators. These may have various side effects. Immunogens used in this project generally have little adverse effects, however we will use previous experience and data in the scientific literature to inform our use. Mice will be monitored appropriately after injection.

Implantation of tumour cells under the skin could cause adverse effects such as significant weight loss, distress or pain. Appropriate analgesia will be provided after injection and otherwise when necessary to reduce pain. The animals will be closely monitored after tumour implantation and Schedule 1 killed if any adverse effects are observed which exceed moderate severity. The subcutaneous tumour models is well established at our facility, and staff are expert at monitoring animals during the procedure and minimising adverse effect.

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

Experimental design, execution and publication will be done in line with the PREPARE and ARRIVE guidelines and in accordance with published best practice for use of animals in cancer research (British Journal of Cancer (2010) 102, 1555 – 1577).

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

We will reference online tools NC3R's Experiment Design Assistant for experiment design, statistical analysis to predict the numbers of animals used in the study.

For each experiment, we will produce a written protocol. In this the numbers of mice used and the procedure they will undergo will be details, all study report written according to the ARRIVE guidelines.

We will keep up to date following NC3Rs news and courses, attending NC3Rs and other 3R focussed events regularly offered at our institution. We have also signed up for the NC3Rs newsletter.