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NON-TECHNICAL SUMMARY

Regulation of inflammation in tissue repair and regeneration

Project duration

3 years 6 months

Project purpose

- (a) Basic research

Key words

diabetes, wound healing, tissue repair, inflammation, chromatin

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

To understand how diabetes and ageing impact inflammatory cell memory and response.

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?

Wound healing is impaired in the elderly and in people with certain conditions, such as diabetes. Chronic non-healing wounds are the leading cause of non-traumatic amputations in the developed world today and are a significant burden on the health care system. Understanding the underlying causes and molecular mechanisms contributing to chronic wound formation is critical to future therapeutic development.

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

We intend to produce new information about how inflammation is normally controlled during wound healing, and how it goes wrong in ageing and diabetes. We will also identify new factors that can potentially be used therapeutically to help problem wounds heal better. Although we will be studying this in the context of skin, much of what we learn may apply to other tissues and organs such as the intestines, heart, liver, or lung. We will disseminate this information at national and international conferences, on social media, and in scientific journals.

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

The work has the potential to inform new lines of research and development of new therapeutic strategies to treat chronic inflammation and impaired wound healing. This will benefit academics in the fields of gene regulation, inflammation, and wound healing, and more broadly across the regenerative medicine subfields in the short-term (likelihood is within a year). Research focused on understanding whole genome structure and dynamics, a field called bioinformatics, will benefit from our data on how genes are controlled following injury by integrating this information into a model that facilitates the building of a predictive framework in the medium-term (likelihood within a few years). There are numerous potential benefits for medicine, particularly with regards to a better understanding of factors impacting wound healing and inflammation in the longer term (5-10 years). Understanding how chemical modifications to DNA regulate gene expression to maintain and/or direct differentiation and behaviour of inflammatory cells in wound healing may also be exploited to develop both diagnostic assays as well as new therapies in the longer term (5-10 years).

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

We will collaborate across the Establishment to support and facilitate other studies by providing access to genetic "toolkit" resources such as mouse and cell lines that our lab has. Likewise, collaborations with clinicians will provide human samples and data to help narrow down relevant avenues to pursue in our animal studies. We will aim to focus on factors relevant to human wound healing in order to produce the largest impact with our studies.

Dissemination of new knowledge will be done through presenting at national and international conferences, social media networks, and print and online journals. In addition, large sequencing datasets will be uploaded to accessible online databases such as GEO. This will be particularly useful when data is "neutral" and difficult to publish in standard journals. We will report our findings using ARRIVE (Animal Research Reporting of In Vivo Experiments) guidelines ensuring their utility to other researchers.

Species and numbers of animals expected to be used

- Mice: 800
- Rats: 200

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 mice and rats because they are the least sentient animals that closely model human immune cell function during wound healing. Non-mammalian immune systems and wound healing processes are not well conserved with mammals. Mice and rats are excellent model organisms for this purpose as they have a highly conserved inflammatory response at the molecular and cellular level, and provide very powerful genetic tools to dissect the key factors controlling wound healing. We need to use both mice and rats because each rodent provides a better model for the two types of diabetes (type 1 and type 2). Rats are much better models for type 1, whilst mice are better models for type 2 due to their specific physiology. We will mostly study type 2 diabetes, as that is the most prevalent type of diabetes in humans, but we will want to evaluate if our results from our studies in type 2 are valid in type 1 as well. Likewise, we will mostly focus on ageing mice, as they are more economical and have more genetic models available. Mice and rats are equally valid models for ageing studies, however, we may want to validate our findings from mice in rats as well, as rats have longer lifespans than mice so are more useful to analyse long term effects of interventions.

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

Approximately 300 mice and 100 rats will be bred under this project under Protocol 1. Under Protocol 2, approximately 125 diabetic, 125 aged and 250 healthy control mice and 30 diabetic, 30 aged, and 30 control rats will be wounded (or used as unwounded controls) over the period of the licence using 1cm or smaller diameter excisional wounds to the dorsum (back). Mice and rats will be given

anaesthesia and analgesics (pain killers) at the time of surgery. Excised skin taken at the time of wounding (the disk removed to create the wound) will be used as unwounded control skin for each animal. Animals will be housed individually to avoid interference with wound healing until the specified endpoint (usually 1-2 weeks). Animals will be humanely killed and wound tissue collected to obtain samples during the inflammatory and healing phases of wound healing. Wound tissue will be used for isolation of wound cells or histology and staining (wax or frozen tissue analyses). Bone marrow will also be isolated from killed animals and immune cells will be cultured for analyses. Animals will be closely monitored for any signs of distress during the experimental period.

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

Some animals may experience moderate pain during the wound healing process, which could lead to weight loss or abnormal behaviour. Analgesics will be used to control pain as far as possible. We monitor the animals regularly to check for these and other indications of discomfort. Typically these effects are only seen for a couple of days.

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)?

We expect these procedures will be mild to moderate severity in 95-100% of animals, based on past experience. If there are indications that the severity has exceeded this, for example if the animal acts as if they are unwell, which occurs in 0-5% of animals, vet advice will be sought or the animal will be humanely killed.

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?

This study is designed to understand how diabetes and ageing impact inflammatory responses during tissue repair and regeneration, and the molecular mechanisms by which these responses are regulated. We will analyse what the diabetes- and ageing-induced changes are, and if changes to cells in the diabetic or aged environment are reversible, either by transplanting the cells into a healthy/young environment to see if changes can be reversed over time, or by treating the cells with factors that can potentially reprogramme them. We will be utilising human patient samples where we can (for cell culture experiments), however, we have to use animals in this study in order to manipulate cells in the physiological environment (in real wounds). Physiological changes in real wounds are complex and

cannot be copied in cell culture conditions. Immune cells act differently at different stages of healing and respond to unknown cues in the wound environment. In addition, immune cells are continuously recruited to wound tissue from other areas of the body, such as the spleen and bone marrow. Thus we must obtain immune cells from real wounds to learn how they are controlled and test factors we think may be used to therapeutically heal wounds better. At this stage of research however, there are practical and regulatory reasons why we cannot take this research straight into humans.

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

Human patient-derived cells in culture and organoid culture. These can model some aspects of wound healing but are not a substitute for the real tissue environment. We must ultimately study immune cells in a fully functioning immune system.

Why were they not suitable?

We need to investigate the effects of diabetes or ageing in a physiological setting, as culture conditions do not accurately model the conditions in the body.

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 needed based on previous studies. These studies were focused on the same types of experiments that we are planning, for example gene expression analyses and high throughput sequencing. We also consulted with a professional statistician to ensure our estimates were correct for the work planned.

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

We adhere to the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines for experimental planning. We also use experiment planning tools, such as the NC3R's Experimental Design Assistant and previously published studies to determine the minimum number of animals needed for each experiment.

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 use our extensive past experience, advice from colleagues, statisticians, and animal facility staff to optimise the number of animals needed. It is also important to ensure proper detailed training of research assistants and students who may be performing experiments.

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.

The animal models we have chosen for this project are the STZ mouse and rat model of diabetes type 1 and the mouse model of type 2 diabetes: the Leptin receptor deficient mouse, as well as aged rats and mice. This choice is based on how well they mimic diabetes and ageing in humans, their sensitivity (they are the least sentient models we can use for our study), how well-characterised they are, and our expertise. We will primarily use mouse models of type 2 diabetes, as this is the most prevalent type of diabetes (90-95% of diabetes cases are type 2) and the Leptin receptor deficient mouse is an excellent model of this disease and has been very well characterised. However, we will also want to validate our findings in a type 1 model. The STZ model of type 1 is best tolerated and is most efficient in the rat, so we will most likely use rats to validate our findings. We may need to use STZ mice if we need a particular genetic background that can only be found in mice. The mouse and rat models we have chosen are proven models used in most wound healing studies, so will provide the most robust data for our studies and will be the most useful for other researchers in the field as they will be directly comparable. The animals will be given anaesthesia so they will not feel anything when they undergo wounding. They will also be given pain killers so when they wake up they will not have any discomfort. They will be watched closely to make sure they do not show any signs of being in pain or becoming ill. If they appear to be in pain or appear unwell, veterinary advice will be sought.

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

Animal models that are less sentient, such as frogs, do not have similar enough physiology to humans to mimic the problems caused by ageing or diabetes during wound healing. We cannot terminally anaesthetise the rats or mice during our study because they need to undergo wound healing for 1-2 weeks so we can study the course of inflammation during healing.

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

All animals will be monitored post-treatment by both animal husbandry staff and lab members daily. For ageing animals, added enrichment will be added to cages (cardboard boxes, wood blocks, cardboard tubes) to mitigate risks of increased aggression. For wounding experiments, additional pain relief can be provided if any concerns arise, under veterinary advisement. All lab members will keep up to date

with the field and any new refinement opportunities. Our 20+ years of experience in this field means we are highly efficient with the standard techniques used, which will benefit the welfare of the animals and significantly reduce harms.

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

Resources published on the NC3Rs website, for example in their in vivo resource library, contains the most up to date best practice guidance for all of the in vivo techniques we will be using. Best practice in animal husbandry and welfare is carefully monitored by the Establishment and training (both online and in person) is regularly required by all staff.

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

The NC3Rs website is an excellent source of information to stay relevant. In addition, the animal facility conducts regular workshops and has a monthly newsletter that keeps users up to date. Any new advances in methods to implement the 3R's will be adopted during the project as appropriate.