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G. Non-Technical Summary (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed programme of work clearly using non-technical terms which can be understood by a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary will render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at www.gov.uk/research-and-testing-using-animals.

Word limit; 1000 words

Project Title	Regulation of inflammation in wound repair
Key Words	wound healing, inflammation, diabetes, ageing
Expected duration of the project	3 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
No	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
No	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);
No	(d) protection of the natural environment in the interests of the health or welfare of man or animals;
No	(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work;
No	(f) higher education or training for the acquisition, maintenance or improvement of vocational skills;
No	(g) forensic inquiries.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Diabetic patients and animals have severely impaired skin wound healing and humans often develop chronic skin wounds. This also can occur in elderly patients. Complications from chronic wounds costs the health service over 10% of their annual budget and is devastating for these patients' lives. By comparing factors in skin wounds from diabetic and aged animals with normal skin wounds, we can begin to understand what is important for efficient skin wound healing and how to promote impaired skin wound healing. In particular, immune cells from diabetic and elderly patients and animal models are altered and inhibit skin wound healing. However, this process is poorly understood and the key mediators that control these cells are not known. Many pro-inflammatory factors are much higher in diabetic and aged chronic skin wounds compared to normal skin wounds, but whether they are causative or a consequence of altered immune cells is not known.

Our objectives are to (1) determine differences in how genes and other factors contribute to altered immune cell behaviour in diabetic and aged rodents and how this impacts skin wound healing (2) test whether we can manipulate these factors and reverse the altered cell responses in diabetic and aged skin wounds. The results of this study will be important in future therapeutic development.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

The results of this project are intended to: 1. Identify the underlying molecular mechanisms that contribute to altered immune cell behaviour. 2. Contribute to scientific knowledge related to chronic skin wounds. 3. Identify potential new therapeutic strategies to promote healing in diabetic and elderly humans. The potential benefits of this study include the development of potential drug, gene and cell based therapies to aid patients with chronic skin wounds and reduce the need for limb amputation.

What types and approximate numbers of animals do you expect to use and over what period of time?

Over a 3 year period: 1600 mice (approximately 1000 for breeding purposes and 600 for experimental procedures) 160 rats (approximately 100 for breeding purposes and 60 for experimental procedures)

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

This study is designed to understand how inflammation is controlled in a normal skin wound and what might be different in a diabetic or aged skin wound. The wounding protocol is considered moderate severity and the breeding and bone marrow transplant protocols are considered mild severity. In some experiments we may need to use oral administration or injections of drugs to elicit a particular condition. This is well-tolerated in rodents and will only cause momentary discomfort. Wounds of 10 mm diameter or less will be made on the back and the effect of manipulating different factors during wound healing will be tested. Adverse effects include stress and discomfort following the procedure. In some cases the substances we treat the mice with may interfere with wound healing or cause the animals to become ill unexpectedly. To mitigate these possibilities, after surgery, animals will be provided with pain relief and monitored closely (twice a day) for any signs of distress. Distress in rodents after this type of surgery is very rare, however, if there is any indication of suffering we will seek veterinary advice or make a decision on the condition of the

animal using established scoring criteria. If the animal does not show improvement after 24 hours, the animal will be humanely killed. In some studies we will exchange bone marrow from one mouse/rat to another mouse/rat in order to determine the effects of the diabetic or aged environment on how bone marrow cells develop and behave. To do this we condition a recipient with a dose of radiation that will allow for the donor's bone marrow to replace the original. The animals do not feel anything during the radiation treatment and they are given replacement bone marrow following their treatment, so they should only feel mild and momentary discomfort during this injection. These animals will be monitored twice a day for radiation sickness or anaemia. This is rare, but if they show signs of this then they will be humanely killed. Animals may also lose weight due to some damage to their digestive tract from the radiation, however, this should only be transient. Animals will be weighed every other day to monitor this, and any animals showing abnormal weight loss will be humanely killed. After 8 weeks' recovery, blood sampling will be performed and should only cause momentary discomfort. Some animals may be wounded to track cells to their wounds. They will receive anaesthesia and pain medication as described above. Strategies to minimise adverse effects due to our treatment, as well as minimise the number of animals needed for these studies include testing the effects of the factors we are putting on the wounds in cell culture first. In this way we will be able to identify the most promising candidate factors without using animals. This will reduce the chances of inducing an adverse effect, and reduce the number of animals needed to accomplish the objective. Animals will be humanely killed at the end of each study.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

We have to use animals in this study because understanding how immune cells interact with skin wound healing in a pathological environment must be studied in the complete physiological setting in order to get an accurate picture of this process. Mice and rats are the least sensitive animals that accurately mimic disease in humans (in this context we understand 'sensitivity' to be the animal's ability to sense and respond to the world around them). The use of animals that can express fluorescent proteins facilitates the tracking of cells. We cannot use humans for these experiments because we would not be able to modify their genes nor track the cells from the bone marrow.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

By conducting experiments in cell culture (in vitro) first, we will identify many of the factors that may regulate immune cells. We will also test potential therapeutic treatments in cell culture models of wounds first. To plan for our animal work, we have consulted a statistician to establish the number of animals required for each study. Also, where possible, we will use two wounds per animal to reduce the number of animals required in balance with refinement. In addition, live imaging experiments will be used, which will allow repeated measures to be taken and thus reduce the number of animals.

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Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

By keeping up-to-date with the latest scientific literature and conference proceedings, as well as consulting with colleagues that have expertise in our area, we will continuously refine our experimental design. The species and models we have chosen are based on how well they mimic diabetes and ageing in humans, their sensitivity (they are the least sensitive models we can use for our study), how well-characterised they are, and our expertise. 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, and do not show signs of recovery in 24 hours, they will be humanely killed.