Home Office

NON-TECHNICAL SUMMARY

Factors Regulating the Skin Immune System in Health and Disease

Project duration

5 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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Skin, Inflammation, Regulation, Tolerance, Immunology

Animal types	Life stages
Mice	embryo, neonate, adult, juvenile, pregnant

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 identify factors which regulate skin immune responses and to understand how these factors fulfil this function.

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?

Chronic inflammatory skin diseases such as psoriasis and atopic dermatitis/eczema are very common, can have a debilitating effect on people’s lives and they place large burden on healthcare systems. Skin inflammation, exemplified by redness and rashes, is also a very common reaction to drugs and infections, which in some case can be serious, yet such reactions remain poorly understood.

The skin is a crucial barrier between the internal body and the environment, and as such it is home to a vast array of immune cells which are tasked with preventing infection by microbes. However, these same cells must remain unresponsive to the harmless microbes living on the skin and to harmless environmental substances which are constantly being encountered. Therefore, skin immune cells must be strictly regulated to prevent inflammation and immune responses occurring inappropriately. Regulation is also key to ensure that inflammation is resolved appropriately after an infection has been cleared, as failure to do so will result in inflammatory skin disease.

Despite the prevalence and impact of inflammatory skin diseases, our fundamental understanding of the factors causing such disease are not well understood, and little is known about how inflammation is regulated and resolved. This lack of understanding impedes the development of new therapies, as appropriate mechanisms to target are not known.

In the past 10 years there have been huge improvements in treatments for moderate to severe psoriasis and atopic dermatitis however even these therapies do not cure disease and they also have significant side effects, they don’t work in all patients, and some patients develop reactions to the therapies themselves over time. Therefore, there remains an unmet clinical need for new therapies.

This project will identify factors regulating the initiation and resolution of skin inflammation and will examine mechanistically how these regulatory factors work. This will improve our fundamental understanding and will also identify novel factors which can be used in the future as targets against which new drugs can be developed for treating inflammatory skin diseases. The regulatory factors identified may also have roles at other sites in the body, so this work may be applicable to inflammatory

diseases at other tissue sites, particularly those sites which contact the external environment such as the gut and lung where the regulation of inflammation is also critical.

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

This work will lead to publications detailing how skin inflammation is regulated in mice and how the resolution of inflammation is controlled. It will identify important factors in the regulatory process which may be future targets against which to develop new therapies to treat inflammatory skin diseases. Importantly, it may also determine factors which are not useful targets for regulating skin inflammation.

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

In the short term this research will benefit immunology researchers by providing a better understanding of factors which regulate inflammation in the skin. These findings may also be directly relevant for other body sites, particularly the gut and lung where there is direct contact with the environment and microbes. Therefore, this work will impact researchers studying inflammation in the lung and gut.

This research will also benefit dermatologists as it will provide new understanding of the factors contributing to skin inflammation. In the long term we anticipate that this research will lead to the development of new therapies which will allow dermatologists to have improved treatment options for their patients.

The project will benefit drug developers at pharmaceutical companies as it will deepen our understanding of factors that regulate skin inflammation and will identify new factors. This will identify new targets against which therapeutics can be developed for the treatment of inflammatory skin diseases. These targets may also be relevant for the treatment of inflammation at other body sites, so this research may have wide ranging implications for treatments for inflammatory disease across many body sites. Identifying novel therapeutic targets benefits the pharmaceutical industry as they can develop therapies which target these factors, leading to new therapies.

The research undertaken will determine how the skin immune system is regulated which will determine new therapeutic targets. We anticipate that this advance will lead to the development of new therapies for inflammatory skin diseases such as psoriasis and atopic dermatitis. Therefore, in the long term we anticipate that this research will lead to improved treatment options for patients with inflammatory skin diseases.

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

We will disseminate the findings of this research through publications in open access journals, press releases and presentations at national and international conferences. We will ensure that the findings of this work reach the widest possible audience by presenting the research at conferences attended by both basic researchers and health care professionals.

We will collaborate with researchers in dermatology to begin to translate the findings of this research, and we will also collaborate with researchers examining inflammation at other body sites to determine if the factors that we find to be important for the regulation of the skin immune system are also involved in regulating the immune system in the lung or gut.

In addition to publishing findings identifying factors that are involved in regulating the skin immune system, we will also publish any findings which determine factors that do not regulate the skin immune system. This is critical to ensure that other researchers can gain insight from our data and will not repeat the same approaches that we have already used unnecessarily. It is also essential information for the development of therapeutics, as it may aid the identification of factors which do not regulate the skin immune system but, may regulate the immune system at other body sites, which may be an advantage for some therapies.

Species and numbers of animals expected to be used

- Mice: 7500

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.

This project requires the use of mice so that we can identify factors which cause (or prevent) disease and can distinguish these from factors which are merely changed in the diseased state, but do not directly affect the disease. The use of mouse models of disease also allows us to determine how these factors work, which is important as this can highlight other factors involved in disease. The use of mice allows the control of variables such as genetic differences and environmental exposures which cannot be easily controlled for in humans.

Working with human samples allows the identification of factors which are altered in disease relative to healthy individuals however, such approaches cannot distinguish between factors driving or preventing disease, and those which are an outcome of the disease, unless clinical trials are undertaken. To undertake clinical trials, prior knowledge and data are required suggesting that the anticipated outcome will be beneficial to patients. For example, we need to have evidence that a drug treatment targeting a particular factor will likely have a beneficial effect for patients, and it is not usually ethical to treat patients with a drug treatment that we suspect will induce or significantly worsen disease. Therefore, research is required in disease models before we can progress to translational research and clinical trials.

Whilst some research can be performed on human skin samples taken from volunteers and grown in the lab, these suffer from an inability to recapitulate human inflammatory skin disease (such as psoriasis or eczema) due to the inability to recruit immune cells from the blood stream into the skin tissue, which is an important stage in the initiation of inflammatory skin disease. Also, although some immune cells can survive for days in the lab, others are unable to survive and do not behave as they would in the body. The use of human skin samples also has drawbacks as there is high genetic variability between individuals and it is difficult to control for additional variables such as the level of exposure to sunlight, environmental chemicals and microbes, and variations in the disease severity

etc. Therefore, to robustly identify factors involved in driving or preventing inflammatory skin diseases, animal models are required where these variables can be controlled.

One of the most well used species for immunology research is the mouse, which has similar immune cells to humans and where well-characterised research tools are available to allow us to study the immune system. Mice are also a particularly good species to use as we can use genetically identical animals to accurately identify the effects of treatments on disease severity, and we can also use existing or newly generated genetically modified strains to allow the determination of the role of specific factors in driving or regulating disease.

In this research we will use adult mice where their immune system is fully developed. This will mirror psoriasis, as this disease largely affects adults. Although atopic dermatitis/eczema is more prevalent in the young, we will also use adult mice for atopic dermatitis models, as we can induce similar immune responses in adult mice to those seen in atopic dermatitis, so this is an appropriate approach which will allow us to interrogate regulators of the disease process whilst ensuring that the severity of our models is minimized.

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

In this research we will use adult mice to model the inflammatory skin diseases psoriasis and atopic dermatitis (eczema). These models involve daily treatments to the skin surface, or injections to induce inflammation which resembles these diseases. This typically occurs by anaesthetising the mice, taking skin thickness measurements using callipers, then treating the skin surface with a liquid or cream, or with an injection into the skin with an agent which modulates a specific part of the immune system. Treatments are typically performed daily for up to 10 days (but usually 7 days or less). In some cases, the resolution of inflammation will be examined by treating the mice to induce inflammation, then ceasing treatment and monitoring the clearance of inflammation.

Prior to, during, or after the induction of inflammation some mice will be treated with immune modulating agents or cells to allow us to determine the effects of each factors on inflammation. This may be via injections or the diet or via the use of genetically modified animals. We will also treat some mice with light radiation to label cells within the skin to allow us to track cell migration into and out of the skin tissue. This may be performed prior to, during or after the induction of inflammation.

Mice will be treated a maximum of once per day and all treatments and measurements will be done under a single period of anaesthesia, unless they can be humanely performed without anaesthesia. On occasion mice will be anaesthetised purely to measure the inflammation in the skin using callipers, as this is cannot be performed accurately without causing distress unless anaesthesia is used. This will be kept to a minimum and each mouse will be anaesthetised a maximum of 15 times in each experiment, although in most cases this will be 7 days of anaesthesia.

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

Psoriasis and atopic dermatitis (eczema) models.

The application of Aldara cream to the skin surface to model psoriasis causes weight loss, typically around 10% of the starting weight, but it does not usually cause dehydration or other overt signs that

the mice are unwell. The local skin becomes reddened, scaly and thickened which are hallmarks of psoriasis. Some inflammation in other parts of the body are observed in this model involving spleen enlargement and immune cell changes, but obvious signs of disease, such as a hunched position are not typically observed.

The administration of the vitamin D3 analogue, MC903 to the skin surface causes atopic dermatitis-like skin inflammation, involving local skin reddening, scaling and thickening and weight loss of typically around 10-15%. Similar to the psoriasis model, we do not expect to observe dehydration, or other overt signs that the mice are unwell in this model.

Skin inflammation may also be induced by treating the skin with immune modulators (cytokines or chemokines), which are small proteins that direct immune responses). We expect to observe reddening, scaling and thickening of the skin at the application site, but we do not expect to see dehydration, or other overt signs that the mice are unwell. Mild weight loss may be observed in this model, typically less than 10%. In some cases we will need to perform tape stripping, where the outer most layers of the skin are removed using adhesive tape. This itself causes mild damage to the outer layers of the skin, resulting in redness, but it is not expected to result in weight loss or any other adverse effects.

Typically ear skin will be treated, but if scientifically necessary then back skin will be treated.

For all of the skin inflammation models, if weight loss nearing 20% is observed, or the mice appear dehydrated, or show other overt signs that they are unwell, the mice will be humanely euthanised. Skin inflammation is expected from around 2 days after the initial treatment, for the duration of treatment and for up to 3 weeks after the cessation of treatment, whilst the inflammation is resolving. Typically, skin inflammation experiments will last for 7 days, so mice will undergo 5 days of inflammation. Where the resolution phase is being examined mice will experience skin inflammation for up to 3 weeks.

The radiation that we use to mark cells in the skin is at the boundary of UV and visible light and has the capacity to cause burns if used from a high intensity source or if the exposure occurs over a long period of time. However, we will use a low power ultraviolet source to minimize the risk of this occurring, and we typically irradiate for 5 minutes. Mice will be checked immediately following irradiation, and the following day for signs of skin tissue damage. In the unlikely event of this occurring, mice will be euthanised.

Treatments with diphtheria toxin can cause weight loss and toxicity if used in high doses or for a prolonged period of time. Pilot experiments will be used to optimise the dosing regimen for diphtheria toxin treatments to ensure that the most appropriate method is used with the least severe side effects. Other procedures such as treatments with cells or immune modulating agents are expected to cause no more than transient distress and no lasting harm.

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)?

These models are to study skin inflammation, so will necessarily cause a number of days (typically 7 days, but up to 3 weeks) of inflammation which will be moderate in severity. Most animals treated will

develop skin inflammation, but around 10% will not because they are either control mice, or the immune modulatory intervention given to them will be successful in reducing the inflammation.

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?

Immune responses involve multiple cell types interacting in the context of a structured tissue architecture complete with a host microbiome. Therefore, although individual aspects of an immune response can be modelled in vitro, to identify novel factors regulating immune responses, models are required where the tissue structure and many types of immune cells are present. Ex vivo human skin biopsies are one approach, but these lack the ability to recruit cells into the tissue, and many immune cells do not survive well in these models. Therefore, the most valid approach is the use of an animal model where all of the relevant immune cells are present and/or can be recruited and the cells interact in the context of the tissue microenvironment.

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

To study human skin, 'skin equivalents' can be used where structural skin cells (fibroblasts and keratinocytes) are grown in the lab and become organised into a skin-like structure.

The other model system that we considered is using human skin biopsies which can be grown in the lab and treated to examine skin immune responses.

Human and mouse laboratory cultures of skin cells will be used where appropriate alongside animal models to allow us to test the effects of each factor on immune cell activity prior to its use in mouse models. This will allow us to replace skin inflammation experiments on live mice (protocol 2 or 3) with laboratory culture models where we will examine the effect of the factor under investigation, on the activity of a specific immune cell type.

Why were they not suitable?

Human skin equivalents are a promising approach however, they currently lack the ability to include most of the immune cells which are the drivers of inflammation and are the main focus of our research. Therefore, skin equivalents are currently not a useful approach to use for this research.

Similarly, the culture of human skin biopsies do not support the survival of many immune cell subsets and non-skin-resident cells cannot be recruited, again limiting the utility of this approach for skin

immunology research.

Some cell culture approaches will be used which will replace some animal use, but it will be necessary to test some factors in animal models as well as in culture systems where there is the full range of cells present in the correct tissue architecture.

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 typically use 6 mice in each experimental group to ensure that we can robustly answer our research questions whilst minimising the number of mice required. We estimate that we will use up to 200 mice a year for psoriasis model experiments for the 5-year duration of the project, which equates to up to 1000 mice. We also require similar numbers for the atopic dermatitis model.

Inducing skin inflammation by the use of immune modulators (cytokines and chemokines) will be used less frequently, therefore, we will use up to 500 mice for these approaches.

To label cells, delete certain cells, or examine how immune regulatory factors work, transgenic mice will be required. Wherever possible we will breed these to give both transgenic, and control mice within the same litter to reduce variables such as cage-to-cage variation. This increases the number of mice generated that are not useful (the incorrect genotype), so increases the number of transgenic mice generated, but it does reduce the variability and therefore reduces the number of mice undergoing procedures. Therefore, we will generate up to 5000 transgenic mice during this project.

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

We consult with statisticians to optimise our experimental design prior to starting experiments and through the project at appropriate intervals.

To reduce the number of mice required, we analyse multiple aspects of skin inflammation in the same group of mice. One example of this is scoring the skin inflammation daily, which allows us to track the severity of inflammation so that the most appropriate day to end the experiment and analyse the skin inflammation is evident.

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

For some mouse strains the most efficient breeding strategy where all mice are useable (have the required transgene or are required controls) is breeding the controls separately to the transgenic mice. However, this approach is not optimal as even maintaining strains using the best practice can result in mutations, and also the mice have different mothers and are separately housed, resulting in multiple unwanted variables. To remove these, wherever possible mice will be bred so there are transgenic and control mice in the same litters. However, this approach can also generate a significant number of unusable mice (e.g. heterogygotes). We will use littermate controls whenever possible therefore, our breeding will not be the most efficient approach, but it will lead to less variable data, reducing the number of mice which undergo procedures.

To ensure that our experiments have optimal sample sizes, power calculations have been performed. However, once we have a better idea of the variability of inflammation in each new strain of mice, we will adjust the power calculation to ensure that an appropriate number of animals is used and therefore mice will not undergo any procedures unnecessarily.

When using new treatments, pilot studies will be performed to optimise the dosing regime, thus ensuring that the optimal protocol is employed, reducing the use of mice.

To reduce the numbers of mice required we will take multiple tissues from each animal, ensuring that a panel of outcomes are measured, so that fewer additional experiments are required.

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.

We will use published skin inflammation models induced using Aldara cream, MC930/Calcipotriol and cytokines such as IL-23. These models work by activating the skin immune system and thus inducing inflammation, rather than causing damage to the skin. The previous best model for psoriasis used immunodeficient mice and involved grafting them with non-lesional human skin from psoriasis patients. The skin inflammation models used here will cause less pain, suffering and distress than this xenotransplantation model as surgery is not involved in the models we will use. The models we will use are also better models of the immunology of psoriasis, as they are driven by host inflammatory responses and are not directed against foreign material, as is seen in the xenotransplantation model.

Any skin inflammation model will need to induce some level of suffering as inflammation is required, but the models that we will use are induced by methods (topical or intradermal treatments) which themselves do not cause lasting pain or harm to the animals.

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

This research is examining factors regulating inflammatory skin diseases. Therefore, models are needed which have a similar immune system and skin to humans to enable the research to be easily translated. Therefore, mammals are required, and mice are the most appropriate species due to their small size and the existing knowledge and tools to modulate genes, cells and immune responses which will allow us to determine mechanisms involved.

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

We will work to speed up taking measurements and applying the inducers of inflammation, so that mice spend the minimum amount of time under anaesthesia. We already check the mice on recovery from anaesthesia, and around 10 minutes after recovery, but we will add in additional checks if required in future.

We will refine our protocols by reducing the number of days that the mice are treated for where our experimental readout is an immune cell type or activity which is important for the induction of inflammation. To determine the optimal day for analysis we will perform pilot experiments and will then choose the shortest treatment time which will enable us to answer the research question.

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

We follow best practice guidelines such as PREPARE (Smith AJ et. al.; PREPARE: guidelines for planning animal research and testing Lab Anim. 2017, 52(2):135-141), those published on the NC3Rs website and in peer reviewed publications such as Morton DB, 2001 (Morton DB *et. al*.; Joint Working Group on Refinement. Refining procedures for the administration of substances. Lab Anim. 2001, 35(1):1-41). We also receive alerts from Pubmed when new skin inflammation papers are published, and we discuss these in fortnightly journal club meetings. In particular, we discuss how the experiments were performed, and if this has any implications for how we perform our inflammation models, with a view to incorporating any refinements in our protocols.

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

We stay informed about 3Rs advances through posters and newsletters sent by the animal facility, and through the NC3Rs newsletters and attendance at NC3Rs symposia. We implement new advances by holding regular group lab meetings dedicated to animal work, where we discuss any issues with the colonies, discuss possible refinements to procedures, and plan how to implement any advances in the 3Rs.