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NON-TECHNICAL SUMMARY

Regulation of immune responses across organs during early life

Project duration

2 years 0 months

Project purpose

- (a) Basic research

Key words

Skin, Lungs, Neonatal, Infection, Allergy

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant 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?

The immune system encounters many challenges throughout life especially in barrier sites located between the outside and the inside of the body, like the skin and lungs. Interestingly, the same mechanisms that are employed to fight infections can be distorted and give rise to allergies. Thus, the aim of this project is to understand how age and successive immune challenges impact on the regulation of barrier tissue immunity during infection and allergic inflammation.

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?

The regulation of the immune system is a complex process. For example, the same immune responses used to fight off parasites such as hookworms and pinworms, are also found to be the basis of allergies. Interestingly, immune responses are regulated very differently throughout life. As such, as individuals grow older, so does their immune system. Early life is considered to be a critical time-window to establish either protective or harmful immune responses. Thus, it is commonly accepted that allergies start to develop and manifest mostly soon after birth. However, the reasons behind that are currently not well known. Therefore, understanding the mechanisms underlying beneficial and detrimental immune responses (while taking into account how immunity is regulated throughout life) is key to help us develop better therapeutic approaches to manage allergies and prevent infection through vaccination. This is particularly important, as 44% of British adults suffer from at least one type of allergy, and this number has been rising over the years. In addition, one quarter of the world human population is affected by parasite infections. Overall, allergies and parasite infections represent a significant burden in human health. Importantly, these conditions also affect animal health. Parasites are widespread in animals, both in the wild and in pet/farm animals. In farming, parasite infections lead to the loss of billions of pounds annually. Although allergies are not so common in outdoor animals, the percenteage of pets suffering from at least one form of allergy is also on the rise, as they share the same environment as their owners. As explained above, the questions being answered by this project are based on the complex interactions between the immune system, the environment and time, overall making it very difficult to study them using alternatives to animal work, such as in *in vitro* or computational approaches. Using well-established mouse models of parasite infection and allergic inflammation will allow us to tackle the complexity of these processes and ultimately translate our findings to human diseases.

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

At the end of this project we expect to discover new mechanisms controlling the balance between beneficial and harmful immune responses during early life. Throughout the duration of this work we will develop new disease models to understand how allergies develop and test current theories on this how and why they became more prevalent in the recent years. Moreover, we expect to define in detail what immune cell populations are critical for starting and regulating the immune responses to parasites and allergens. The approaches used here will also allow us to identify the molecules produced by these

cells. These findings will lead to publications in peer-reviewed journals, dissemination to scientific audiences in specialised conferences and to the wider public in outreach activities.

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

Type-2 immune responses are responsible to help us fight parasites, but can also cause allergies when not regulated properly. In the short term, we expect to establish valuable new models to study type-2 immune responses (both good and bad) during the course of life. This will help to form the foundations of my newly established research group. Also, with time, we expect these models will be used for other researchers in the field. Importantly, allergies affect many organs in the body, so we expect that our findings will also guide research in other scientific areas connected to the allergy field, such as neuroimmunology, cardiovascular sciences and health education.

In the longer term I believe our findings will lead to a better understanding of why allergies arise during early life, and why this has become more prevalent in human populations. Thus, we expect this project to ultimately benefit patients currently suffering from allergic conditions by generating knowledge that might lead to better therapeutic strategies. Furthermore, our data might also bring benefits to the efforts of preventing the onset of allergic reactions in the future. Finally, these findings may also be relevant to animals with allergies, as they share the same environment as their owners and type-2 immune responses have been shown to work similar across different species.

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

This project will allow us to establish collaborations with other basic and clinical researchers. Working together with multidisciplinary teams will allow us to make our results more relevant.

Furthermore, the data generated in this project will be presented both at scientific meetings and outreach activities, aimed at the scientific community and the lay public, respectively. Presenting our data to these different audiences will guarantee that our results are of the highest quality and applicable to the needs of people suffering with allergies.

Finally, we plan to have the full results generated in this project available to the community, either by the publication of peer-reviewed articles (of both successful and unsuccessful experiments) or by uploading the generated datasets in public repositories. It is important to note that our results will be published as open access will be first uploaded in preprint servers, such as www.biorxiv.org allowing for maximum reach.

Species and numbers of animals expected to be used

- Mice: 750

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 are the most versatile model for establishing models of infection and allergic responses. There is an extensive body of literature describing how different immune system components impact both protective and pathological immune responses, with clear translation to humans. Furthermore, the widespread availability of immunological reagents and transgenic lines for mice, allows for multiple complementary approaches to manipulate their immune system, which would not be possible using other species.

We will use neonatal (1 to 5 days post birth), juvenile (5 days post birth until weaning), and adult (post weaning) mice in our experiments, both from wild-type (like the C57Bl/6 strain) and different transgenic mouse lines. Whereas adult mice possess an immune system that has developed to a degree that models the adult human immune system, we will use neonatal or juvenile mice to try and uncover the mechanisms and events shaping the immune system during early life that can have lasting effects on the immune system and the response to infections and allergies.

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

Typically, mice will be infected with parasites and/or be exposed to an allergen via the skin (topical/percutaneous application). In some cases, mice will receive a single or multiple injections containing immunomodulatory substances (e.g., antibodies to block immune responses or to deplete a specific cell type). Normally, mice will be experience 2 or 3 procedures that may each cause short mild degrees of suffering. In some situations, mice may experience moderate degrees of suffering.

To a lesser degree, experiments might look at the immediate immune response in the first few days after infection, allergen exposure, or administration of an immunomodulatory substance. More commonly, experiments will last between 1 or 2 weeks to capture the fully formed immune response to a parasite or an allergen. However, some experiments may last several months in order to evaluate the role of multiple allergen and/or parasite encounters, or assessment of immune memory. These experiments will typically last between 1 and 3 months. For longer experiments (typically not exceeding 3 months), mice may receive multiple doses of an immunomodulatory substance (e.g., once a week for 2-3 weeks), and samples (e.g., blood) may be taken to monitor immune response development over time. All the experiments will end with animals being killed humanely, sometimes under terminal anaesthesia.

Separate from the above experiments, some genetically altered animals will be used only to breed and maintain animal lines.

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

Most of the animals undergoing experimental procedures, even with immunological manipulation, will experience mild, and no more than moderate, adverse clinical signs.

Breeding and maintenance of genetically altered mice with specific deletions in immune function genes or transgenic expression of immune receptors are not expected to exhibit any harmful phenotype, and would be regarded as no more than mild. Our experience working with parasite infection and allergy models suggests that the tissue damage caused by the immune system against the pathogens and

allergens can result in local or systemic inflammation and pathology. This usually manifests as weight loss, the involuntary bristling of fur, reduced spontaneous activity and reduced response to external stimuli. However, in most cases, experimental animals will display mild symptoms and only a small proportion of mice will progress to moderate symptoms. Airway inflammation after using established parasite infection and allergy models can induce in some animals (5-10%) temporary (less than 24 hours) respiratory symptoms resulting in moderate severity limits. Namely, mice may show a increased breathing rate, with deeper breaths and in rare cases may show laboured breathing and reduced activity. However, most experimental animals will not develop beyond mild symptoms. Some protocols will involve general procedures such as restraint, injection or use of anaesthesia. All of these provide the possibility of adverse effects, but none beyond moderate severity. All animals will be humanely killed at the end of each experiment.

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 experiments proposed in this project will not go beyond the moderate severity limit. I expect a majority (around 55%) of animals to experience mild severity after being subjected to the procedures proposed on this licence. The rest of animals (approximately 45%) will experience moderate severity. All the animals in the breeding protocol should not experience anything above the mild level.

What will happen to animals used in this project?

- Killed
- Used in other projects

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?

Infectious diseases and allergies encompass a series of complex interactions between host and pathogen. From the host side, both immune and non-immune cells sense and integrate a variety of signals aiming for pathogen/allergen clearance whilst maintaining tissue integrity. Such complexity is, normally, very difficult to achieve with the use of *in vitro* and *in silico* approaches. Moreover, as this project addresses questions at the whole organism level by studying the communication between immune cell populations from different organs throughout the course of an infection/allergic response (during different life stages), the use of animal models is vital to answer our questions having age and organ of interest as variables. The mouse is ideally suited for the proposed research as the populations involved in the establishment of protective immunity are similar between mice and humans; employing the same immune pathways and relying on the same soluble factors to exert their functions.

The search for replacement options was conducted based on the replacement checklist available at: https://replacinganimalresearch.org.uk/resources/replacement-checklist/. In brief, I searched the PubMed database for in vitro approaches to mainly study the lung responses to allergens (these encompassed cell lines, precision lung cut slice, and primary cell cultures). I also used AI (https://chatgpt.com) to search more broadly (i.e., in these queries, information outside traditional publishing channels were considered) for alternatives to animal research looking at inter-organ communication. These searches were conducted multiple times along August and October 2025, and were accompanied by advice from leaders in the field. It is important to note that the most promising strategy for replacement was the multi-organ "organ-on-a-chip" approach, which is still in phase of development and establishment, it is technically challenge and still requiring solutions. I will, nonetheless, keep updated in the developments in this are and try to implement it whenever it becomes more technically feasible. However, even in this situation, we cannot add the infectious component of my research questions, as the parasites used in this project require a living host.

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

Wherever possible, we will use *in vitro* approaches to establish causal relationships between given factors and immune cells of interest. For instance, we can add inflammatory markers to immune cells in culture and evaluate their response. Although *in silico* approaches are improving, they still cannot predict the outcomes of complex interactions between the immune system and external challenges, in particular when studying the response across different organs. In fact, *in silico* approaches need experimental data to get more robust. This notwithstanding, we will use *in silico* pipelines to direct our studies and select more relevant pathways to study. Finally, given that the experiments planned in this licence aim at intervene on immune responses to investigate the best ways of regulating them, human samples are of limited value. These types of samples are a valuable alternative and we will employ them whenever it is possible, but they will not be applicable to all of our objectives.

Why were they not suitable?

The experiments proposed in this project involve complex processes that require the recognition of external signals (parasites and allergens) by the immune system. This involves not only the activation of immune cells, but also the engagement of structural cells located in the epithelium and endothelium. Moreover, a key aspect of this project is to evaluate how immune responses are regulated across organs, and during different life stages. Therefore, achieving this complexity with added spatial and temporal layers is impracticable using standard *in vitro* techniques or more advanced approaches such as organoids. Human samples are also not feasible for most of the experiments proposed here, as they won't allow us to manipulate immune responses across time and organs.

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?

The number of mice has been estimated based on the extensive experience I gained during my previous work. I have been using mice to model different aspects of immune responses over the course of my scientific training (10+ years), allowing me to accurately predict the numbers of mice to be used in this licence. The final number takes into account breeding strategies for genetically altered mice, and anticipated numbers of planned studies over the course of the license.

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

For all of our experiments, in-bred mice are used to reduce experimental variation, which makes it possible to use fewer animals to achieve statistical significance. For the majority of our studies, mice from the same litters are used for control and experimental mice, reducing variation that can occur due to differences in the microbiota. Overall, our experiments are designed to reduce the number of variables to as few as possible and thereby reduce the number of control groups required.

We are well acquainted to both the NC3Rs experimental design assistant and trained in statistical methods. This helps to ensure that we will be using optimal numbers of mice in our experiments. Randomisation and blinding are included whenever practically possible. Tissue sharing is a major tool we use to reduce animal usage.

A sizeable proportion of our animal use will be related to breeding programmes for genetically altered lines. We will follow the advice of our animal facility staff to optimise breeding, and ensure we do not overbreed. Where possible and appropriate, the use of substances that can target or block immune processes in wild type mice will be employed in order to reduce use of genetically altered mice.

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

A number of measures will be taken to optimise the number of animals used throghout this project. Firstly, pilot studies will be performed whenever preliminary data are necessary, allowing for adjusting and reducing the numbers whenever possible. In addition, tissue sharing is a practice that will be occurring and encouraged not only within my own lab, by through active discussion with other research teams, where we have found opportunities to work together.

The increased use of genetically altered mouse lines has led to more complicated breeding strategies and, as a result, larger mouse colonies. The use of littermates as controls will help to reduce the numbers of animals used both directly (by optimising the use of animals per breeding), and indirectly, by decreasing variability in our experiments. Additionally, when a particular strain is not being used experimentally we work closely with the animal technicians to maintain low numbers of stock animals through optimised breeding strategies.

Finally, I will have regular checks on our mouse usage to ensure we are keeping on track with the proposed number of animals to be used.

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.

Nippostrongylus brasiliensis, a rodent hookworm parasite, provides an excellent model to study skin-lung communication, given the parasite life cycle. The migration of the parasite through the lung induces a classic inflammatory response, followed by an appropriate type-2-mediated repair response, both of which are regulated by immune events happening in the skin. This model allows us to study the formation of type-2 immune responses using a natural route of infection. In addition, the possibility of having consecutive rounds of low-dose infections allows us to study how the immune system adapts to multiple challenges and how this relates to other chronic stimulation conditions such as allergen sensitisation. Over the course of my career I optimised this infection model to ensure that most animals do not present adverse symptoms beyond the mild level. By adjusting the dose of larvae used to infect the animals we are able to minimise animal suffering but still study the pathways of immune cells and tissue damage responses. Importantly, this dose adjustment will also take into account the life stage of the animals used. Finally, we refined a suffering scoring table to allow us to better judge when to humanely cull mice undergoing a protocol.

Experimental allergy models have been instrumental to advance our understanding of human allergic diseases. Throughout the years, different protocols have been established, encompassing different routes of administration, how often the animals are exposed to, and the nature of the allergens. These models have been instrumental in building our knowledge in the mechanisms behind the initiation and aggravation of allergic responses. The deep understanding of type-2 immunity underlying many disease aspects gained from experimental allergy models, has been key for the development of novel drugs and therapeutic strategies to manage allergies in humans. Thus, these models are invaluable tools to study how allergic inflammation is regulated across the skin - lung axis. In this project, we will employ an allergen dosing regimen during a defined timecourse that will very rarely induce symptoms beyond the mild severity threshold.

Mice represent the most appropriate species for in vivo study of immunity, because of the extensive knowledge of their physiology as it relates to humans, the genetic and biological tools available and the ability to be easily bred and handled. For the mouse models that have been used elsewhere in the literature, we will make sure to use their most refined version to minimise animal distress. For the mouse models that we aim to establish within the remit of this licence, we will start from refined individual procedures and continuously refine the full protocol within the duration of this licence. Procedures involve administration of allergens topically or by inhalation, or parasites by injection or direct delivery, and injection of immune modulators to reduce or alter disease progression.

Most of the animals undergoing experimental procedures, even with immunological manipulation, will experience mild, and no more than moderate, severity limits. Doses and timing are carefully managed

such that animals will experience minimal suffering. We are constantly assessing and refining our approaches to ensure robust experimental results whilst minimising pain, suffering or distress. Wherever possible we will analyse mice at the earliest possible timepoint to avoid prolonging their suffering. But it is important to note that the infection model used here is self-resolving and that the allergy model will not normally involve chronic responses.

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

Standard laboratory mouse strains are readily infected with the pathogens or allergens we study via the natural route (e.g., via the skin or the nose). In the mouse, for the models we will be using, the route of infection, site of pathogen development and the immune cell response are similar to that in humans. Unfortunately, none of this can be fully replicated in less sentient species. To my knowledge, no other species of lesser sentience can fully recapitulate these processes as well as the laboratory mouse. Importantly, the aim of this project is to study long and complex immune processes over different life stages and organs. For example, we need to understand how the immune cell populations travel between organs during parasite infections and allergies in order to regulate inflammation. In addition, we want to understand if these cells behave differently in specific life stages. The immune cell compartment in the mouse develops very similarly to its human counterpart, which does not happen in non-mammalian species. Finally, we cannot use terminally anaesthetised animals as we require the mice to develop immune responses to the external challenges, and to analyse the outcomes of these challenges over time and in response to interventions.

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

Animals will be monitored for adverse effects using score sheets previously developed in conjunction with the Named Veterinary Surgeon (NVS) and NACWO. These score sheets have proven to allow for objective measurements of clinical signs associated with adverse effects arising from parasite infections and allergic inflammation, in order to determine when humane endpoints have been reached.

Experimental approaches will be designed taking into account all appropriate provisions to avoid or alleviate suffering, pain, distress and discomfort. This includes the use of anaesthesia, analgesia and pain relievers in all potentially painful procedures. In addition, we will employ, where possible, the least invasive methods for dosing and sampling. Strict humane endpoints will be carefully followed to avoid unnecessary suffering.

Highly specialized technicians will be involved in the daily care of the animals and the health and welfare status of the animals will be continuously monitored. All personnel working with mice will attend ongoing compulsory training sessions for handling and experimenting on mice.

Finally, animals that are more susceptible to the infection model proposed in this licence will be housed in a barrier environment minimising the likelihood of adverse effects.

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

The government animal testing and research: guidance for the regulated community (https://www.gov.uk/guidance/research-and-testing-using-animals)

Morton et al., 2001, Refining procedures for the administration of substances; Laboratory Animals, 35, 1-41

The NC3Rs webpage: https://www.nc3rs.org.uk/

The PREPARE and ARRIVE guidelines: https://arriveguidelines.org, https://norecopa.no/PREPARE

Standard Operating Procedures developed with the animal facility and the named veterinary surgeon.

For the parasite infection and allergy models, my lab members and I will read papers from other groups doing similar experiments, as well as consulting directly with other researchers and collaborators to discuss the most refined procedures.

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

I already have in place an active communication channel with the animal facility, allowing for timely consultation regarding the best practices involved in the models we will use throughout this project. Through discussion with our Named Training and Competency Officers (NTCOs) and Named Animal Care and Welfare Officers (NACWOs) I will keep all the protocols up to date in regards of the 3Rs latest advances.

In addition, my team and I will aim to attend Experimental Design and other relevant workshops organised by the animal facility to ensure we achieve our scientific aims with the minimal number of mice.