



Home Office

NON-TECHNICAL SUMMARY

Immune cross-talk between the oral and distant mucosal barriers

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

Periodontitis, Immune response, Mucosal Immunity, Inflammation, T cells

Animal types

Life stages

Mice

neonate, juvenile, adult, 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?

The aim of this project is to determine immune mechanisms that drive oral inflammation and better understand how oral inflammation contributes to the pathogenesis of inflammation at distinct mucosal barriers. Mucosal barriers are sites that separate the internal body from external environment and include sites such as the gastrointestinal tract, lung and skin.

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?

Periodontitis, is commonly referred to as "gum disease" and the most severe form of oral inflammation. It is the most common chronic inflammatory disorder of mankind; affecting almost 50% of the global population. Moreover, it has been predicted that almost 80% of the global population exhibit less severe forms of oral inflammation, such as gingivitis. Not only is oral inflammation therefore an incredibly prevalent pathology, but periodontitis has been associated with many other systemic diseases. For example, individuals with periodontitis have been shown to exhibit increased severity and/or incidence of rheumatoid arthritis, cardiovascular diseases, Alzheimer's Disease, diabetes, chronic obstructive pulmonary disease (COPD) and inflammatory bowel diseases (IBD). Thus, better delineating the immune mechanisms that contribute to the maintenance of oral health would not only promote the development of better therapies for periodontitis, but may also have implications for the treatment of other inflammatory conditions.

This work investigates the immune mechanisms which reinforce health within the oral cavity, defining how they become un-controlled during oral inflammation. Moreover, our work will outline how immune dysfunction within the oral cavity and initiation of periodontitis can contribute to the development or exacerbation of inflammation at distal mucosal barriers.

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

Two key outputs will emerge from this project that will advance our knowledge of oral inflammation and its systemic consequences. First, information generated from these studies will contribute to our understanding of how immune responses are appropriately controlled at the mucosal barriers of the mouth, yielding key information on important pathways that can go wrong during the development of oral inflammation, and more specifically periodontitis. Secondly these studies will provide some of the first mechanistic insights into the immune-based communication between the oral and distant mucosal barriers, as our approaches will outline how oral inflammation can contribute to extra-oral inflammatory diseases such as inflammatory bowel disease (IBD). We predict that the data generated within these studies will have a positive impact on the treatment of both periodontitis and as well as other diseases such as IBD, providing not only novel treatment options but also suggest mechanisms to stratify patients or identify patients at risk of developing both of these two, hugely impacting disease.

To maximise these benefits data generated from the studies undertaken will be effectively disseminated to the research community, (including academic and industrial researchers, medical and dental clinicians). This will be achieved through; i) publication of data in academic journals; ii) presentation of data at seminars and conferences and iii) through public engagement events. In this way data generated within this project will rapidly inform the work of others and promote scientific discovery within our research community.

There is a key secondary benefit that will also arise from undertaking this work; our work will advance research methods, through refinement and/or enhancement of current methods as well as through the possible development of new transgenic lines.

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

Scientific Community: This programme of work will generate new data, research tools and advance research methods which will be shared with the scientific community with the ultimate effect of promoting scientific discovery. This impact will occur in both the short term - following publication or talks but, overall support scientific advancement worldwide in the long term. Data generated will benefit researchers who focus on immunology and oral and gastrointestinal inflammatory diseases. However, our work will be more broadly applicable to other fields including microbiology, development and systems biology.

Healthcare Sector: Outputs from this research will, in the longer-term, have the potential to impact the healthcare sector, both medical and dental clinicians. This work will provide basic biological insights into the drivers of periodontitis and the mechanisms by which this disease impacts distant mucosal barriers, such as the gut or lung. It is hoped our studies will provide novel therapeutic targets for the treatment of oral, gut and lung pathologies targeted at the very accessible oral cavity. We also hypothesize that our studies could support the development of better mechanisms of patient stratification.

Biomedical Industry: Data generated from this project will also support the biomedical industry, as findings from our research are translated into the clinic. This impact will occur in the longer-term as novel therapeutics and/or treatment strategies emerge from our data sets.

General Public: The importance of oral health is often overlooked in the face of pathologies which drive more considerable suffering or lead more obviously to shorten life-spans or life-quality. Information obtained from this project can be used to engage the general in the vital importance of oral health, highlighting the impact poor oral health, and subsequent development of oral inflammation, could have on completely distal organs.

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

Dissemination of new knowledge: Output from this work will be published in highly visible (high-impact) journals which are read by a wide audience. Following publication of data in scientific papers we will make use of social media platforms to further publicise our work, including contacting daily newspapers and magazines (which have run stories on our work in the past). Alongside this, participants undertaking work on this project will be supported to discuss the work widely, presenting seminars and giving talks both locally and globally.

Collaborations: We already have in place a global network of academic and clinical collaborators that we will engage with as we develop this work programme. Outputs with collaborators will be maximised by regular meetings and data exchanges.

Publication of Negative Data: Although not common we have a strong track-record of publishing negative results. We will continue this whilst undertaking this project, ensuring publication in the most appropriate journals possible.

Species and numbers of animals expected to be used

- Mice: 5000

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.

The studies outlined will utilise juvenile and adult mice, which may be genetically modified. These life stages are employed to examine mice with teeth and/or mice just prior to tooth eruption. Mice are the most appropriate species for these studies as the systems which we are studying here (the immune system at the oral and distal mucosal barriers such as the gastrointestinal tract and lung) are well reproduced between mice and humans.

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

Approximately 70% of the animals utilised on the project will undergo a procedure to investigate oral inflammation and the impacts oral inflammation has on immune health at distal mucosal barriers. To achieve this the following approaches will be used:

Oral inflammation will be induced in mice. This will be via one of three methods, each representing a key tenant of the pathology of oral inflammation. Either (1) periodontitis will be induced by the placement of a silk ligature around the second molar tooth. (2) The oral barrier will be damaged by abrasion with a sterile cotton applicator. Or (3) periodontitis and oral barrier damage will both be induced. For all these procedures mice will be anaesthetised and oral inflammation will result.

Altering the immune response at distal mucosal barriers. Here we will assess the impact of oral inflammation on immune function at distal mucosal barriers, such as the lung and gastrointestinal (GI) tract. We will initially explore impacts of periodontitis on gastrointestinal immunity. To investigate alterations in gastrointestinal immune responses one of three approaches will be used, each will allow us to examine the impact of oral inflammation on a distinct aspect of GI immune responsiveness.

Prior to, and/or, during these procedures the immune system may be manipulated, through application of reagents to target specific cell types (e.g. through use of antibodies or tracking agents) or through

transfer of cell populations.

In addition to these procedures, mice will be used in this project for breeding, and for provision of cells and tissues for ex vivo studies

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

Animals may experience mild adverse effects such as temporary stress or brief pain and discomfort. Temporary stress may be induced by a brief period of restraint, for example in order to administer an injection. Mice may experience a brief period of pain and discomfort following induction of oral inflammation, through any of the three methods outlined, as well as following blood sampling. In these instances the stress and discomfort will be transient, specifically ranging from a few minutes to less than 12 hours.

Weight loss will occur after the following procedures (1) ligature-induced periodontitis, (2) administration of antibiotics, (3) generation of chimeras and (4) gastrointestinal bacterial challenge. In all cases this will typically be in the range of around 10%, with this weight loss being transient, for example ranging from a) following ligature placement weight is usually recovered within 48hours to b) following chimera generation weight is usually regained by 3 weeks.

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 studies outlined in this project will result in the following proportions of animals actually experiencing the following severity ratings:

Sub-threshold – approximately 20%

Mild – approximately 30%

Moderate – approximately 50%

What will happen to animals at the end of 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?

(1) There are no good in vitro or in silico models able to reconstitute the complex interactions occurring between oral tissue, host immune cells and microbiota and extra-oral mucosal barriers. The aim of my research is understand how immune responses are appropriately controlled at the oral barrier, and how immune dysregulation at the oral barrier can impact immune responses at distal sites; in vivo models provide a unique window to probe this.

(2) The mouse is a good model to understand the human oral barrier, as the immunological network and tissue structure is similar in mice and man and the pathophysiology of oral inflammation similar in mouse and man.

(3) Inbred mouse strains will be used, allowing uniformity between animals and experimental repeats.

(4) There is considerable use of knockout and transgenic animals, which are not available for other species. Transgenic mice are already generated, are well characterized and available for use to assess gene and cellular functions.

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

In vitro: Approached that include cell-lines or co-culture systems (where multiple cell types are cultured together). We utilise such approaches as these to i) inform the animal studies to be undertaken and ii) further validate targets and pathways identified in animal models.

Human samples: we have an active research program obtaining and examining gingival samples from human dental clinical cohorts. With detailed clinical information and patient meta-data such samples can be used to correlate immune phenotype with disease characteristics.

Neither of these approaches can replicate the complex environment of the oral barrier or model the distal interactions with the gastrointestinal tract and therefore cannot replace the use of animals.

Why were they not suitable?

Although the above approaches are valid platforms for research investigation neither fully replace animal models for the following reasons: i) Our studies in human can only be correlative and will not mechanistically link cause and effect, which can only be achieved in animal models. ii) our research questions require complex in vivo systems to precisely define the complex interactions between heterogeneous cell populations in their tissue microenvironment and also how they may impact responses at sites distal to the initial challenge. To date these cannot be accurately modelled in vitro.

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?

Our group has extensive experience with the methodologies and approaches outlined here and of running projects of a similar scope. Consequently, estimates of animal numbers are based firstly on previous experience with the models to be utilised and the types of data generated, and secondly with careful consideration of the experimental design.

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

Throughout the duration of this licence, we will continue to consider the design of each experiment (as is required as a PPL holder). As we generate new data sets these will be used to further refine our experimental design.

For this application we have utilised data sets already generated from experiments which have led us to undertake this project. These data sets, along with our research objectives, have been discussed with the institute statistician, allowing us to get specialist advice on the types of experiments that will be undertaken and the nature of the datasets that will be collected. This has supported development of appropriately designed experiments which will yield biologically relevant and interpretable data with the fewest numbers of animals possible. We will continue to liaise with this expert as our project develops and new data is generated.

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

Throughout this project, optimal animal usage will be achieved by the following means:

- 1) Utilising the most effective and efficient means to breed transgenic mouse lines. This will be achieved by consulting local and commercial experts. In addition we employ an electronic stock management tool to monitor our colonies allowing real-time checking and maintenance. In this way we will minimise numbers of animals bred whilst still achieving allowing us to undertake experiments from which we can generate reliable data.
- 2) Pilot studies will be employed to optimise experimental conditions when we are developing new approaches.
- 3) We will maximise the amount of data that can be gathered from a single animal by using the latest technologies and most efficient tissue processing methodologies.
- 4) We will archive all tissue possible from each experiment. We also make our banked tissue available to our collaborators.
- 5) We will discuss our findings and share data generated with collaborators and the scientific community (for example through depositing large datasets in online data repositories). In this way we will maximise the scientific knowledge than can be obtained from our animal studies.

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.

This project involves the use of models of mucosal barrier inflammation. In both cases we will employ the most refined models available to address our experimental objectives. For example;

(1) For the establishment of periodontitis in mice we will use the ligature-induced model of disease. This model results in 100% disease incidence and the disease is also temporally identically in each mouse; meaning treatments and experimental manipulations can occur concurrently. This model is also widely employed with others in the research community and has the additional advantage that disease development can be halted – by simply removing the ligature. Finally this disease occurs over an acute time frame, meaning period of time for which the mouse is showing signs of inflammation will be kept to the minimum possible to address the research question.

(2) For the gastrointestinal (GI) challenges, the models to be used have been selected to drive well-defined immune responses in the gastrointestinal tract, leading to acute inflammation which will resolve, i.e. none of the methods employed lead to the establishment of chronic inflammatory reactions and as such, mouse suffering is kept to a minimum. All GI challenges employed have been well optimised in terms of doing route, dosage and time post-administration in order to induce a robust and consistent response whilst causing the least harm and suffering to experimental animals.

Where we seek to administer reagents to experimental animals we utilise the most refined route of administration possible. Furthermore we always seek to minimise the numbers of doses of a treatment in order to achieve our objective, this may involve pilot studies to identify an optimal dosing regimen.

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

We cannot replace these studies in mice with studies in less sentient species, such as insects or fish, to achieve our objectives. Such species lack a defined oral barrier which is comparable to humans. Moreover, they lack the complex immune found in higher order species.

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

1) Appropriate animal handling techniques will be employed and updates, which further reduce stress and discomfort, employed as they are developed.

2) After procedures animals will be closely monitored in line with the refinement controls listed with each experimental step, for example this will involve detailed monitoring of weight and condition. Where transient weight loss is expected following some procedures, soft food/mash will be provided on the cage floor.

3) All animals will have environmental enrichment in cages and whenever possible, not singly housed.

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

We will base our approaches on the best published practises, for example those endorsed by the NC3Rs.

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

Myself, as well as those employed by me and working under this license, will stay informed on recent advances in 3R approaches by staying up to date with NC3Rs recommendations and developments. This information from the NC3Rs is obtained through interaction with their website, local seminars, reading their updates and published bulletins and also through liaising with their local representative. We also continue to discuss further refinement opportunities with the NVS and NACWO and through interaction with collaborators and the wider scientific community at conferences, workshops and seminars.