Home Office

NON-TECHNICAL SUMMARY

Investigating joint immunity 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

Key words

Joint immunity, Autoimmunity, Infection

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?

To improve our understanding of how immune cells work in joints in response to autoantibody, infection and inflammation, and to determine how responses of immune cells are influenced by the microbiome (the bacteria that live in the gut).

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?

Over 10 million people in the UK have arthritis due to a variety of disorders and more than 30% of people in England suffer from chronic joint pain. Joint pain can intrude everyday life, affecting the ability to work, to move free from pain, and live independently. Joint immune cells are the first responders to injury and infections and they release substances, called cytokines, to induce joint inflammation and pain. But our understanding of their function and interactions is unclear, and will be the focus of this work. In addition, we also don’t know how immune cells in the joint are influenced by the microbiome.

This information is important to develop better treatments for inflammatory joint diseases, such as rheumatoid arthritis and psoriatic arthritis. These chronic diseases are currently incurable, affect patients for decades, and have a significant impact on their lives and ability to work, with substantial health-economic implications. Our work will shed light on the mechanisms by investigating how local and systemic immune challenges affect immune responses in the joint, potentially identifying new treatment strategies.

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

This project aims to better understand how immune cells within joints respond when the body encounters infection or inflammation—either locally in the joint or elsewhere in the body (systemically). We also want to find out whether gut infections or changes in the gut microbiome (the community of bacteria in our intestines) can affect inflammation in the joints. In the short term (1–2 years), we expect to map out the basic immune responses in joint tissue under different conditions and identify any early signs of gut-joint communication. In the medium term (3–4 years), we aim to uncover specific pathways or immune cell types that are influenced by gut health or systemic infection. This will help us understand whether targeting the gut or the immune system more broadly could reduce joint inflammation. In the long term (5 years and beyond), the findings could inform the development of new therapies for joint diseases like arthritis by highlighting novel ways to calm harmful inflammation—either by modifying immune responses or by addressing gut health. This knowledge may ultimately reduce the need for animal use in future studies by narrowing down the most promising therapeutic targets early.

We will ensure that the information generated is widely disseminated. Our group has a strong track record in producing publications containing our work, including in the highest impact journals.

In addition, we will also produce ‘methods’ papers or book chapters to ensure our experience of best practice is available to the field.

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

Joints are affected by autoimmune inflammation (eg, in rheumatoid arthritis) and by infections and they are also ‘barometers’ of systemic inflammation – with joint swelling and pain being common symptoms of systemic viral or bacterial infections. Our work will help understand which immune cell contribute to joint defence, inflammation, and pain. This will also help us understand whether joint inflammation can influence immune activation in other organs (short-medium term benefit). These will together lead to discovering novel therapies targeting at joint swelling and pain, and will benefit patients suffering from joint pain (long term benefit).

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

We will maximise our outputs via research collaborations and dissemination of our work by presenting in scientific conferences and publishing in scientific journals. We will also try to present unsuccessful work in the scientific conferences and local communities for the benefit of the scientific community. Our datasets will go to open source to prevent repetition.

Species and numbers of animals expected to be used

- Mice: Wild type and genetically modified mice: 4,100.

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 use mice in our research for several compelling reasons:

Mice share approximately 90% of their protein-coding genes with humans, including many genes related to the immune system. This makes them a valuable model for studying immune and infectious diseases. Research in mice has frequently identified critical pathways involved in human diseases, as documented extensively in scientific literature.

The availability of genetically modified mouse strains is a major advantage. These modifications allow researchers to investigate the role of specific immune cell types by, for example, removing genes essential for the development of certain cells. Advanced genetic techniques can target gene deletions or alterations to specific cell types or control them spatially and temporally using harmless chemicals or

light. Additionally, some mouse strains are engineered to have fluorescent immune cells, enabling real-time visualization of immune cell movements within complex tissue environments.

Mice have a well-established history in research, including widely used models of arthritis, such as the collagen-induced arthritis model. These pre-existing models provide a strong foundation for studying immune responses in disease contexts.

We will mainly use 8-20 weeks old mice because the life stages represent patients who develop autoimmune arthritis, such as Rheumatoid Arthritis.

Taken together, these unique features make mice an invaluable model organism, particularly for assessing and comparing human-relevant immune responses in joint-related diseases—capabilities that are challenging to achieve with other model systems.

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

We will use genetically modified animals, for example, mice that are either deficient in, or have too much of an immune cell type. Most of these genetic modifications do not cause any symptoms in homeostasis (the healthy, unchallenged state).

In some cases we will change the immune system of the mouse by generating "bone marrow chimeras". This is done by giving irradiation to remove the bone marrow immune cells, followed by the introduction of genetically modified donor bone marrow. This allows mice to be generated that lack specific immune cells or immune signalling molecules without making a new mouse strain, avoiding lengthy breeding strategies. The lengthy breeding strategies have potential harms in wasting a lot of animals to generate a new mouse strain, which is against the principles of 3Rs. Following irradiation, mice may temporarily show reduced appetite and are at increased risk of infection. They will be weighed and monitored for abnormal behaviours and food sweeteners/supplements offered to increase oral intake if required. They will also be assessed for evidence of infection and treated appropriately if needed.

Mice will be exposed to joint inflammation (arthritis) by introducing collagen and/or substance (antigen or adjuvant) that causes inflammation subcutaneously (under the skin), intravenously (into the vein), or intraarticularly (into the knee joint). Mice will be assessed for their pain sensation by measuring the threshold to withdraw their legs when knee joints are pinched gently (behavioural test). Mice will also be assessed for the extent of inflammation through imaging technique (e.g. MRI, PET or optical imaging, intravital imaging etc).

Mice will be injected with substances that induce immune responses (subcutaneous, intraarticular, intravenous, or intra-peritoneal (into the abdominal cavity)). The effects on tissue immune cells will be assessed across joints and different organs. Mice are regularly monitored post-challenge.

Mice will be exposed to infection (such as salmonella) by introducing bacteria orally in water or into the stomach by a thin tube, called a gavage needle, that is gently inserted into the mouse's stomach through its mouth.

Mice will experience blood sampling from various surface veins such as tail vein or saphenous vein. The recommended guidance will be followed to ensure that a maximum of 10% total blood volume

(TBV) will be collected on any occasion and no more than 15% TBV within a 28 day period. This won't have any adverse effect.

Female mice will be made pseudo-pregnant and embryos will be implanted either non-surgically or surgically under general anaesthesia. Mild, short-term effects may include stress from handling, recovery from anaesthesia, and post-surgical discomfort, managed with appropriate care. Non-surgical methods are minimally invasive. Female mice will undergo superovulation by receiving hormone injections to stimulate the release of multiple eggs, followed by humane culling at the appropriate time to collect oocytes.

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

The expected impacts on animals vary according to the protocol.

For protocol involving arthritis, penetrance of collagen induced arthritis or collagen-antibody induced arthritis varies with strain and genetic background and is often difficult to predict. Mice may lose weight and may become hunched. However, experience gained using this model has shown that mice with inflamed paws move and forage well and we would not expect more than 20% of mice to reach moderate severity before the end of the experiment. Lameness is not expected and some animals reaching to moderate severity may experience joint pain, which will be treated with analgesia (e.g. Buprenorphine in the drinking water). Mice will be typically analysed in the early time point, which is within 1 week after the onset of arthritis. Mice will be assessed for their pain sensation by measuring the threshold to withdraw their legs when knee joints are pinched gently (behavioural test). Mice will also be assessed for the extent of inflammation through imaging technique (e.g. MRI, PET or optical imaging, intravital imaging etc).

For protocols involving challenge with pathogen or pathogen-associated molecule, mice may experience weight loss, piloerection (hair stands on end), hunched posture and reduced movement. These models are short-lasting, and animals are typically humanely killed 1-3 days after the infection.

For protocol involving intestinal infection, mice may experience weight loss (up to 15% of body weight), diarrhoea, and some abdominal pain. They may also become hunched and show reduced movement. The active phase of our models last around 7 days. After this, the infection is cleared (eg, salmonella). They may also develop pain and this will be treated with analgesia as needed.

For protocol involving irradiation, mice may have weight loss of 15% compared to pre-experimental weight taken at the start of the study. They may also show clinical signs including piloerection, hunched posture, lethargy, abnormal rapid breathing, or diarrhoea.

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)?

Mild 50%

Moderate 50%

Severe 0%

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?

We need to use animal models to understand how tissue immune cells in the joint respond to immune challenge and infection. Especially, we want to understand how immune cells in the joint are influenced by local and systemic immune challenges. This process cannot be recreated using cells in a dish (in vitro) because the local structure and microenvironment of the joint is lost and hence impossible to assess the interactions of different cell types in a dish.

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

We have continued to develop a number of assays to replace and reduce animal use, including:

1. In vitro assays using mouse and human cell lines. We can test the toxicity of drug candidates using cell lines.

2. Ex vivo assays using joint explant taken from knee joints of mice. This can be used as an organoid for a variety of experiments.

3. Use of human tissues. We have obtained ethical permission to use human joint specimens obtained from osteoarthritis patients undergoing replacement surgery or removal of joint tissue. The use of human joint samples may help replacing the use of disease models in mice. We are also working with an academic surgeon to obtain human joint specimen from post-mortem.

Why were they not suitable?

The assays and tissue sources that we use for in vitro and ex vivo studies are useful, but they cannot completely re-create the complex tissue environment of joints and many different cell types and cell interactions that occur in vivo. This is particularly important for our project, as in many cases we are trying to assess how a systemic autoimmune disease status or infection affects immune cells in the joint. These questions cannot be addressed without an in vivo animal model. Furthermore, when considering how joint immune responses are influenced by the microbiome, in vitro cell stimulation is limited in what we can specifically assess.

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 estimated number of animals required for this project is based on experience gained from other similar work conducted by the group. Approximately 800 animals are required per year and 4000 for the life of the licence.

To calculate the number of animals historically required for specific experiments, we have collaborated with a bioinformatician with expertise in statistics. Data from observed variations in historical experiments conducted by other labs working on similar projects and from published studies were used.

For new challenges or models not previously used in our lab and without published data, we will conduct small pilot studies to ensure ethical practices and avoid unnecessary harm to animals.

We added 50 mice to accommodate the embryo recipient females and 50 mice for superovulation.

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

Our experimental design will adhere to the NC3Rs' experimental design guidance and experimental design assistant, supplemented by local statistical expertise. Randomisation and blinding will be implemented as needed, with randomisation achieved through appropriate methods.

To further reduce variability, we will standardise procedures through laboratory Standard Operating Procedures (SOPs) for sample collection. In-house training will be provided to ensure consistent tissue collection practices, including proper dissection techniques, minimising post-mortem delay, and maintaining optimal storage conditions. These measures aim to minimise systematic variation and enhance reproducibility.

Additionally, we include control groups in all experiments, ensuring they are appropriately age- and sex-matched and, where possible, cohoused to minimise intragroup variability caused by microbiome differences. Where possible, we apply blinding throughout experimentation and analysis to reduce bias.

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

To optimise animal use, we actively encourage tissue sharing within the group. A tissue-sharing scheme is in place where all naive wild-type animals and most experimental animals are made available to other lab members for studies focusing on organs or tissues outside the primary scope of the original research. Additionally, we collect and store extra tissues (by fixing or freezing) for future use, maintaining thorough documentation to facilitate use by other group members for method optimisation or training purposes.

We also strive to maximise the utility of animals that are surplus to the requirements of the original experiments by offering them to other researchers to address additional biological questions. For instance, we have used the establishment's 3Rs email list to share surplus animals with other researchers.

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 joint infection, inflammation, and autoimmune models included in our license have been carefully selected for their relevance to the human diseases under investigation. In every case, we use the least severe protocol necessary to address the specific research question. For immunisation and pathogen challenge models, we employ the lowest effective dose required to elicit the desired immune response. Similarly, we minimise experiment durations, tailoring them to the aspect of the immune system under study. For example, innate immune responses can often be assessed in very short experiments, sometimes lasting as little as two hours.

Challenge models are designed to closely mirror human therapy or disease, employing routes of administration that are directly relevant. For instance, intraarticular injections to the knee joint are used to mimic therapeutic injections in human patients. Animals are routinely provided with environmental enrichment, and whenever possible, we house them in groups to reduce distress.

To further reduce animal use, we employ bone marrow chimera models instead of breeding new genetically modified mice with a specific gene deletion. In this approach, host cells are depleted through irradiation, and donor cells are introduced to repopulate the immune system within the host. This method allows us to investigate specific immune cell types without generating new mouse models or undertaking complex breeding programs, thereby minimising the number of animals required. Breeding new knockout strains requires multiple generations of animals to breed and bone marrow chimera can significantly reduce the number of animals required, which contribute to the principle of 3Rs.

Female mice will be used as embryo recipients due to their well-established reproductive biology and suitability for genetic studies. Pseudo-pregnancy will be induced through a natural and low-stress

method. Embryo transfer will be performed using minimally invasive non-surgical techniques where possible, or surgical methods under general anaesthesia with appropriate analgesia. These approaches are refined to minimise pain, distress, and lasting harm. Superovulation will be induced via hormone injections to increase oocyte yield for embryo production. These methods are refined to minimise pain, distress, and lasting harm.

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

Our project focuses on studying immunity in organs such as joints, and we will exclusively use adult mice. Embryonic mice are unsuitable for this research because their immune systems and organs are immature, leading to immune responses that differ significantly from those of adult animals. Additionally, the structural cues within embryonic organs differ from those in mature tissues, further limiting their applicability.

Non-mammalian animals are also unsuitable for this work due to significant structural and functional differences. For instance, many lack synovial articular joints, a key feature of the joint we study. Moreover, their immune cells are less developed, with distinct receptors and responses compared to mammalian immune cells. These differences make non-mammalian models inadequate for studying human organs and diseases.

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

We take several measures to reduce stress in mice, including providing enriched environments and housing them in social groups. For oral administration of substances, we ensure they are as palatable as possible by using flavoured options such as jelly, paste, or milkshake liquids. We will give analgesia through drinking water or orally via a syringe to reduce the discomfort when needed.

To minimise the severity and duration of our protocols, we monitor clinical parameters and endpoints frequently. Examples include tracking weight, piloerection, and hunched posture. This ensures adherence to humane endpoints. We use protocol-specific score sheets, such as those for joint inflammation, which document parameters like swelling of paws.

We also reduce animal distress by combining procedures when feasible. For instance, if multiple substances need to be administered simultaneously via the same route, they are combined into a single procedure. We also use non-aversive handling (tunnel and cupping), habituation and acclimatisation to procedures, which will reduce stress.

Specific protocols require additional care to address potential discomfort. For example, when generating bone marrow chimeras, mice may experience reduced appetite and increased infection risk following irradiation. Similarly, in intestinal infection/arthritis models, mice may lose weight. In these cases, we weigh the mice daily and increase monitoring frequency if weight loss accelerates unexpectedly. Sweetened substances are also provided to encourage oral intake and maintain nutritional balance. Diet gels will be given routinely for collagen antibody-induced arthritis model since they tend to lose weight 4 days after antibody injection.

Our lab continually evaluates and refines procedures to minimise discomfort for animals. Team members holding personal licenses under the project license will meet regularly to review and update operational practices. These reviews will lead to improvements, such as reducing procedure durations without compromising the quality or performance of the experiments.

We will use lipopolysaccharide (LPS), which is a molecule found on the surface of certain types of bacteria, from the same company to reduce different batches having different effects due to contamination of the endotoxins.

Non-surgical embryo transfer will be used where possible to reduce invasiveness, pain, and recovery time. When surgical methods are required, animals will be given general anaesthesia and appropriate analgesia to minimise discomfort. All procedures will be carried out by trained personnel to ensure consistent, humane care.

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

We will use the published guidelines to inform experiments, such as the ARRIVE and PREPARE guidelines: http://journals.sagepub.com/doi/full/10.1177/0023677217724823

And guidance from the Laboratory Animal Science Association (LASA): https://www.lasa.co.uk/current_publications/

We will also follow the guidance available via NC3Rs publications and newsletters.

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

We are subscribed to the local NC3Rs email list and regularly review updates and resources on the NC3Rs website. Additionally, we receive newsletters and bulletins from our Named Information Officer and access the latest practical guidance from organizations such as the Laboratory Animal Science Association (LASA), the Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA). These resources ensure we stay informed about the latest recommendations and advancements in animal research techniques.

We also attend rheumatology meetings and conferences, such as the annual congress of the European Alliance of Associations for Rheumatology. These events provide opportunities to learn about and adopt innovative methodologies that can further refine and improve our experimental approaches.