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NON-TECHNICAL SUMMARY

Implications of therapeutic use of complement activation modifying monoclonal antibodies on infectious and non-infectious inflammatory diseases

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Microbial infection, Inflammatory disorder, Complement activation, monoclonal antibodies

Animal types	Life stages
Mice	Adult
Rats	Adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 evaluate new therapeutic technology that will help in the treatment of microbial infection and reduce the inflammatory response associated with the activation of the immune system.

A retrospective assessment of these aims will be due by 28 November 2030

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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 complement system is a well-defined protein system in our blood which has an important part of our immune defense mechanisms. This system participates in the recognition and killing of invading microbes. Activation of the complement also triggers the inflammatory response. Uncontrolled activation can cause tissue injury and the development of some inflammatory diseases such as ARDS (Acute Respiratory Distress Syndrome). The Development of inhibitors that block the complement system in the blood will open a new avenue for novel approaches and strategies for the treatment of inflammatory disorders. Unfortunately, using complement inhibitors may increase the risk of infection with microbes, hence it is important to develop a new therapeutic tool that reduces the possible risk of infection. We will introduce a new therapeutic approach for treating microbial and fungal infections using specific antibodies produced in mice and rats. These antibodies will be evaluated for their therapeutic benefits in mouse models.

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

ARDS is one of the major complications of SARS-COV-2 infection that was responsible for increasing the rate of mortality among COVID-19 patients. It is now very well known that activation of the

complement system in lung tissues has a major role in developing ARDS. Our work will evaluate how complement inhibition will improve lung function in a mouse model of ARDS induced by LPS or acid. In addition, our results will provide a better understanding of how the immune system behaves towards invading pathogens, especially in case of complement deficiency. We will also study how the complement system interacts with different microbial pathogens. The knowledge that we will add on how the immune system interacts with various pathogens will help to develop new strategies for the treatment of bacterial and fungal infections.

We will generate highly specific monoclonal antibodies that target conserved and functionally critical bacterial surface antigens, such as outer membrane proteins, polysaccharides, or virulence-associated factors (factors that help the pathogen to damage host cells). These antibodies will be engineered to exhibit high binding affinity and avidity, ensuring robust and selective targeting of the bacterial pathogen while minimising non-specific binding. Our monoclonal antibodies will facilitate complement activation through the classical pathway, promoting efficient complement cascade amplification, and increased deposition of complement molecules on the bacterial surface. This enhanced opsonization (labeling) will support phagocytosis by immune effector cells, such as macrophages and neutrophils, and drive complement-mediated bacterial lysis via the formation of the membrane attack complex (MAC). Additionally, these antibodies will be designed to overcome specific bacterial immune evasion mechanisms. For example, they will target bacterial proteins that inhibit complement activation, disrupt capsule polysaccharides that shield bacteria from immune recognition or neutralize factors that interfere with immune cell function. By counteracting these defenses, the monoclonal antibodies will significantly improve the efficiency of bacterial clearance by the host immune system. This work offers a promising alternative to traditional therapies. This work is expected to generate significant interest within the scientific community and can be readily disseminated through publications in high-impact factor journals, ensuring broad visibility and advancing the field of infectious disease research.

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

In the short term, the outputs from this project will be communicated to academics working in the field of immunology, where we will assess both the benefits and the harmful effects of complement activation and address the delicate balance between complement activation and down-regulation in health and disease. We will also introduce a new complement-mediated therapeutic approach for the treatment of microbial infection that will open a new window for novel research themes to target multidrug-resistant bacteria. In the long term, we will introduce a new and well-defined study in the field of complement immunology that will help to better understand how the immune system fights microbes, and how to avoid the harmful effects associated with uncontrolled activation of the immune system.

In response to this global need, therapeutic antibodies are gaining traction as a promising approach for the treatment of infectious diseases. Unlike traditional antibiotics, therapeutic antibodies can target specific pathogens with high precision, potentially reducing the risk of off-target effects and resistance development while providing new tools to manage both acute and chronic infections. Despite these efforts and advancements, only 26 monoclonal antibodies targeting bacterial pathogens have progressed to clinical trials, and only three have received FDA approval. This limitation is mainly due to the high diversity of bacterial strains and the ability of microbes to develop new mechanisms to resist opsonisation and phagocytosis by the immune effector cells. Our new engineered monoclonal antibodies are designed to be unaffected by these ever-changing resistance mechanisms. In the short

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these antibodies will be tested in vitro to evaluate it is efficacy, in the medium term, we will test these antibodies in an animal model, and the data will be published to the scientific community as a new therapeutic approach. In the long term, these antibodies will be tested in clinical trials alone or in combination with other antimicrobials to treat multidrug-resistant microbes. bes.

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

Our group has close collaboration with experienced Senior Scientists in our field in the UK and worldwide where we discuss our new findings to increase the benefits for the scientific community. We also participate and present our work in many scientific meetings within the UK or overseas. We publish our new findings in high-impact factor scientific journals. Unsuccessful approaches will also be discussed with our collaborators and will be mentioned in our publications. Sharing animal tissue from our well-designed experiment with our collaborators will help to maximise the benefits.

The generated antibodies can be employed in a range of in vitro applications, including Western blot, ELISA, and flow cytometry (FACS), and will also serve as valuable reagents for in vivo studies. To maximize their impact, we will disseminate our findings through oral and poster presentations at international scientific conferences and symposia, ensuring engagement with the broader research community. Additionally, we will publish our results in widely recognized, high-impact factor journals to facilitate knowledge exchange and advance the field. To support collaborative research efforts, we will make these antibodies available to colleagues working in infection and immunity and will provide comprehensive characterization data, including binding affinity, specificity, and functional validation, upon request.

Species and numbers of animals expected to be used

- Mice: 2700
- Rats: 30

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 will use adult wild-type mice for the infection study. In addition, we will use both mice and rats for the production of antibodies. Mice and rats have long served as the preferred species for biomedical research animal models due to their anatomical, physiological, and genetic similarity to humans. Advantages of rodents include their small size, ease of maintenance, and short life cycle. Rodents also have similar immune responses to infection and disease.

Only adult animals will be used to establish the disease models because the immune response at that stage captures the essential traits of many bacterial infections and tissue injury in humans.

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We will use adult mice and rats for the monoclonal antibodies protocol because the animals at that stage have a well-developed immune system that will initiate an immune response. This immune response stimulates B cells (cells that are responsible for antibody production) to differentiate and develop into plasma cells producing antibodies.

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

In mouse models of microbial infections:

- Adult mice will be purchased from commercial suppliers in the UK at the age 7-8 weeks.

- "We will begin using the animals in our experimental work approximately 1 to 2 weeks after their arrival, allowing them sufficient time for acclimatization.

- Mice will receive a specific bacterial/fungal dose, or it is toxin via intranasal (through the nose) or oral administration as well as by injection.

- Mice will be observed for disease progression by monitoring the signs and the symptoms of illness. These symptoms include hunched posture, stary coat, decreased activity, and lethargy.

- Immunomodulatory and/or complement inhibitors therapeutics could be injected at any time before or after the infection.

- Blood samples will be taken from a superficial blood vessel before and at different time points post-infection to assess the bacterial load in blood to avoid any complications that may increase the severity of disease progression beyond the expected severity limit.

- At the end of the study, all mice will be euthanised (humanely killed) then tissue samples will be collected for pathology.

In the case of the ARDS model:

- Mice will receive a specific dose of LPS (lipopolysaccharide) or diluted HCL (hyrdocholoric acid) via intranasal (or by injection in case of LPS only)

- Mice will be observed for the symptoms of ARDS. The symptoms of ARDS include general features of animal illness such as hunched posture, starry coat, and decreased animal activity in addition to other specific symptoms of ARDS such as labored and rapid breathing.

- Immunomodulatory (Agents that decrease / increase the immune response) and/or complement inhibitors therapeutics could be injected at any time before or after the infection.

- Blood samples will be collected from a superficial blood vessel before and at different time points post-infection to assess the inflammatory mediators in the blood.

- At the end of the study, all mice will be euthanised (humanely killed) then tissue samples will be collected for pathology.

For monoclonal antibodies production:

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- Mice/rats will be immunised (S.C or i.p) with a specific recombinant antigen (recombinant antigens are antigens produced in cell culture to avoid using the whole organism). Animals will be immunised by injection with an antigen emulsified with an adjuvant (an agent that is used to increase the immunogenicity of weak antigens)

- Animals will be boosted by injection (S.C or i.p) every 2 weeks (with either antigen emulsified with an adjuvant or with an antigen in PBS) for 6 to 8 times until a high antibody level in blood is detected.

- Blood samples will be collected from a superficial blood vessel to measure the antibody levels

-Animals will be euthanised (humanely killed) and spleens will be collected.

At this stage spleens from immunised mice will be collected then antibodies-producing cells will be isolated in a cell culture laboratory. The spleen cells will be then merged with specific cells to enhance the survival and increase the productivity of the antibodies. By this technique, we will be able to isolate specific antibodies that can help to bind and kill microbes.

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

In the mouse model of acute respiratory distress syndrome (ARDS), a robust inflammatory response occurs shortly after infection where mice show signs and symptoms that might reach the severe severity limit. Animals will show symptoms such as stary coat, piloerection, lack of grooming, and hunched posture as well as a reduction in their activity within the cage, respiratory distress, and lethargy. A decrease in body weight by up to 20 % will also be observed.

Mice will be immediately euthanized if they fail to respond to external stimuli, indicating a critical decline in physiological function. Additionally, mice will be euthanized if they exhibit clinical signs consistent with the severe severity limit for a continuous period of 12 hours. This time frame is essential for capturing the pathophysiological hallmarks of ARDS. These hallmarks include lung injury, significant leukocyte infiltration into pulmonary tissues, increased alveolocapillary permeability, and excessive mucus accumulation within the lung parenchyma.

Animals that lose 20% of body weight associated with clinical symptoms will be euthanised. Animals that lose up to 20% and start to recover (no clinical signs) will not be euthanised.

In mouse models of infection, mice will show signs and symptoms of moderate severity limit including a reduction in their activity within the cage and a decrease in body weight. Animals will be closely observed as frequently as necessary to ensure that the severity limit is not exceeded. If these signs persist for over 12 hours or if an animal begins to deteriorate at any time, it will be immediately killed.

Immunisation of mice or rats either subcutaneously or via intraperitoneal route has limited side effects and we are not expecting any change in the animal behaviour. These side effects include swelling, redness, and inflammation at the site of the injection.

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)?

1. 75% (moderate) + 25% (mild) (Protocol 1).
2. 75 % (severe) and 25% moderate (protocol 2).
3. 75% of the mice (mild) + 25 %moderate (Protocol 3).
4. 75% of the rats (mild) + 25% moderate (protocol 3)

In summary,

62% of mice will be under moderate severity limit, while 24% and 14% will be under the severity limit of mild and severe respectively.

What will happen to animals used in this project?

- Killed

A retrospective assessment of these predicted harms will be due by 28 November 2030

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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 study how uncontrolled activation of the immune system can mediate tissue injury in some inflammatory diseases such as acute respiratory distress syndrome (ARDS), a lung injury that is always associated with the over-activation of the immune response. This disease involves the recruitment of inflammatory cells into the lung tissue, activation of the complement system in the lung, and the production of inflammatory cytokines (Cytokines are small proteins that act as chemical messengers in the immune system) that cause lung injury. These complex interactions cannot be achieved by any other model that does not include animals.

In addition, we need to study the complex interaction between the innate immune response (natural immune response) and different pathogens. This involves a complex interaction between immune cells, plasma proteins, the blood vessel walls (vascular endothelium), and organ-specific cells that cannot be modeled using ex vivo (using animal tissues in cell culture) or in vitro (in test tubes rather than in animals) systems.

For the production of monoclonal antibodies, the use of animals is essential and cannot be replaced because we need to isolate antibody-producing cells from the spleen and fuse them with tumor cells to

produce an immortal cell line called a hybridoma.

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

Before animal experimentation, extensive in vitro studies using different techniques to assess the ability of different proteins from the immune system to bind bacteria, such as ELISA and FACS analysis techniques. Opsonophagocytosis assays (engulfment of bacteria by white blood cells isolated from human blood) will be performed to identify which strains of the pathogen will stimulate the targeted pathway of the complement system. Assessment of the functional activities of the complement inhibitors (complement inhibitors are agents that stop complement function) will be completed first in vitro using ELISA and FACS analysis (techniques used for analysis) to evaluate the specificity and efficacy of these complement inhibitors. We considered the possibility of limiting the infection experiments to ex vivo studies using blood from human volunteers as a source of complement and cells. Ex vivo studies do not reproduce the course of the infection in a multi-organ model (i.e in animals).

Most animal-free alternative methods for antibody discovery rely heavily on synthetic libraries. While various formats such as scFv, Fab, and VHH can be explored, concerns about library diversity remain. Additionally, these synthetic libraries tend to be very costly, making them inaccessible to many laboratories. In contrast, traditional immunization cycles naturally enhance antibody binding affinity and stability through the natural immune selection process. Antibodies generated via in vitro methods often suffer from sequence liabilities that can lead to reduced stability. We evaluated alternative animal-free approaches for monoclonal antibody production, such as phage display technology. The phage display technique has been successfully used to generate monoclonal antibodies. This method often yields antibodies with suboptimal binding affinity to target cells due to limitations in affinity maturation and selection processes. In addition, it requires additional engineering steps to enhance the affinity and stability of the produced antibodies. Even though this could produce antibodies that still have binding affinity issues. Since we are looking for highly specific antibodies with a maximum binding affinity that will be used later in clinical trials, non-animal alternatives were not a suitable option for us. High-affinity antibodies are critical for effective pathogen neutralization, immune system activation, and therapeutic success.

Why were they not suitable?

It is necessary to study microbial interaction with the immune system in a whole mammalian organism to appreciate the impact of medical treatments on:

a) The spread of the bacteria between different organs in the body

b) Tissue pathology in various organs

c) The effects of the progressive escalation of immune responses

In addition, we need to assess the degree of lung injury because of uncontrolled complement activation in some autoimmune disorders such ARDS. This study requires the tissue to be exposed to different complement components and recognition molecules for a prolonged period of time to achieve tissue injury. In this model, severe lung injury must be induced to mimic the critical situation in humans

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which is normally associated with massive infiltration of inflammatory cells and activation of the complement system with the initiation of cytokine storm. Unfortunately, there is no available ex-vivo model for such complications.

Using animals remains necessary because their immune systems are better equipped to produce antibodies with the required specificity and affinity. This underscores the need for animal models to ensure the therapeutic antibodies generated are suitable for medical applications.

A retrospective assessment of replacement will be due by 28 November 2030

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 long-term experience in animal work and using good experimental design helped us to estimate the number of animals that will be used in each experiment. We also consulted with experienced scientists in our field and within our group. In addition, we consulted a departmental statistician who gave us advice and help whenever needed. Our previously similar animal models published in high-impact factor scientific journals were also a valuable guide to estimate the number of animals that will be used in this project.

For antibody production, we aim to generate antibodies against a diverse set of antigens derived from bacterial, viral, and fungal pathogens. The proposed protocol will focus on the production of antibodies targeting antigens from 10 distinct pathogenic microorganisms. For each microorganism, three specific antigens will be selected, resulting in a total of 30 antigens. Based on prior experience (from my previous PPL), we will use 6 mice/antigen, leading to an estimated total of 180 mice. Additionally, three antigens of human origin will be included in the study (3 antigens × 6 mice = 18), bringing the total number of mice required to approximately 200.

We will also generate monoclonal antibodies against mouse and human antigens. A total of 10 antigens from human and mouse sources will be selected, with each antigen group consisting of 3 rats; a total of 30 rats will be used for this study.

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

Whenever possible, we perform preliminary studies using smaller numbers of animals. We also try to adjust our work to use only one control group that can be used for different experiments. We use the smallest possible experimental number of animals for each experiment being very careful that this does not affect the accuracy of the results. To calculate the smallest number of animals that we can use, calculations based on advanced statistics and mathematics in addition to other published results, were taken into consideration. Online tools such as experimental design assistant (EDA) from the NC3Rs website were used to perform sample size calculations. To increase the quality, reproducibility, and translatability of our animal studies, we will follow PREPARE guidelines. Animal tissues from different experiments will be kept frozen and/or embedded in paraffin blocks that can be used later if more tissue analysis is required.

For monoclonal antibody production, our strategy will be based on using 3-4 mice per group for immunisation of animals. After that, spleens will be collected to produce hybridomas and the selection of the specific antibodies. If successful, we will not use more mice. In some cases, we succeeded in isolating the required number of monoclonal antibodies from the first batch. In other cases, we had to immunise the second group and increase the time between the immunsations steps to allow production of highly specific antibodies. We will also consider immunisation of some animals with 2 antigens to reduce the total number of mice or rats used in the production of monoclonal antibodies.

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

We always run pilot experiments using a small number of animals to assess the feasibility of the study.

In some experiments, we will use bacterial toxins instead of using the bacteria. This will help to minimize the side effects where mice will receive only the calculated dose of the bacterial toxins that show the symptoms and the signs of disease without exposing animals to the bacteria. We will run pilot experiments to test the potency of the bacterial toxins so a large stock of bacterial toxins will be purchased to avoid repeating this step every time we buy a new batch.

We would not start animal work in large groups until we have preliminary positive and encouraging data. Tissues from pilot experiments and major experiments will be frozen or fixed in paraffin blocks for future use. During monoclonal antibody production, we will use 3 animals in case of immunisation with potent immunogenic proteins (proteins that stimulate the immune response). This number will increase to a maximum of 6 mice when using antigens that have a weak immune response.

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A retrospective assessment of reduction will be due by 28 November 2030

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

All the animal models of infection in this work are designed to induce the required severity limits necessary to mimic the disease progression in humans. All the necessary procedures will be taken to achieve the highest level of welfare to the animal starting from good husbandry and animal care before starting the experimental procedures. We will use the most refined mouse model of infection for each pathogen. During the experimental procedures in the infection studies, all the necessary requirements will be taken to cause less stress to the animals during the experimental procedures. e.g. In the case of i.n (intranasal) infection, we will use brief anaesthesia to reduce animal stress and maximise the volume of the dose being delivered. We will use small gauges and fine needles to minimise trauma in the case of intravenous and intra-peritoneal drug administration. Mice will be monitored for any unexpected signs and symptoms of disease progression to avoid exceeding the allowed severity limit. Mice will be euthanised (humanely killed) at early endpoints to avoid exceeding the allowed severity limit.

In mouse models of ARDS, mice will be infected with LPS from E. coli or acid (diluted HCL) to induce ARDS. A highly purified form of LPS with the lowest concentration of protein and RNA (genetic materials) contaminants will be used. In this model, we will use the lowest possible concentration of LPS or acid to induce lung injury. Also, mice will be monitored closely to avoid the disease severity exceeding the allowed severity limit. Mice will be monitored for signs of disease progression including stary coat, piloerection, lack of grooming, hunched posture, decrease in body weight, and a reduction in their activity within the cage. Mice will be also monitored for respiratory dysfunction such as rapid breathing, shortness of breath, or laboured respiration.

During the monoclonal antibodies production protocol, We will immunise mice and rats with antigens emulsified with any of the commercially available adjuvants. Some antigens and/or adjuvants may cause local inflammation in the skin. The irritation is mainly due to the high concentration of the adjuvant. For a better outcome, we will decrease the concentration of the adjuvant (less than 50%) to avoid ulcer formation. Subcutaneous (S.C) injection of adjuvant will be under brief anaesthesia to reduce the risk of leakage of the adjuvant inhe skin that may lead to the formation of skin lesions and skin irritation. The site of injection will be changed every time to avoid skin irritation. We will also use the smallest possible size of needles, minimal numbers and frequency of repeated injections. We will use commercially available adjuvants with a strong safety profile that effectively induce an immune response while causing minimal skin inflammation.

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

We need to study the immune response towards infection caused by the bacteria or its toxins and how the immune response might cause tissue injury as a result of uncontrolled activation of the complement system. This will require animals to be exposed to the insults for a relatively long time (days or weeks) and this cannot be done in anaesthetised mice. In addition, using immature life stages of animals is not a good choice to study the immune response, especially since immature developmental stages of animals are likely to have an immature immune system and therefore will provide results that cannot be extrapolated to reflect the responses expected in mature animals.

Production of monoclonal antibodies requires animals with a mature immune system to elicit a proper immune response when immunised with different antigens. Animals at immature life stages will not produce the immune response required for the production of antibodies.

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

Before major studies, we will perform a pilot experiment. Once we optimise the conditions and get the experiments to work on a small scale, we will perform the major experiments. This will help to avoid the unnecessary use of large numbers of animals. Tissues from pilot experiments will be also kept to be used as controls for future work. Mice will be closely monitored for any sign of disease progression. Animals will be provided with accessible and palatable food supplements if needed to minimise weight loss. Animals that are unwell may be less likely to explore new supplements, so food supplements will be offered a few days before the challenge to acclimatise the animals to it.

Most importantly, animals that will be purchased from external suppliers will be housed in cages of 6 mice or 3 rats and will be allowed an acclimatisation period of 7 days from arrival before they are used for experimental procedures.

Any pain or stress will be diagnosed as early as possible, and animals will be culled by humane methods (schedule 1 method) if necessary. S.C injection of adjuvants and antigens will be done under brief anaesthesia to reduce the risk of leakage into the dermal layer of the skin that may lead to the formation of granuloma and skin irritation. We will also use the lowest possible concentration of the adjuvant required to immunise the animals in this project. Needles will be used once during the injection to minimise the pain.

The use of analgesics is not recommended in this study, as their pharmacological effects could interfere with the experimental outcomes. Analgesics may obscure the clinical manifestations of disease progression, thereby hindering accurate assessment of pathological symptoms. Furthermore, their use could confound the evaluation of the therapeutic efficacy of the antibodies under investigation, making it challenging to distinguish their specific effects.

A proper environment including shelter, tunnels, and a comfortable resting area will be provided to the animals. We minimise mice suffering via careful and skillful husbandry and handling.

All clinical signs and manifestations will be scored when possible in scoring sheets. These scoring sheets will be kept and discussed after each experiment to refine our procedures.

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In the case of immunisation to produce monoclonal antibodies, we will reduce the concentration of the adjuvant to 20%, this reduction significantly reduces the inflammatory reaction (in our previous studies) at the site of the injection without affecting the immunogenicity of the injected protein. In case of skin inflammation, intrasite gel or a NSAID (e.g. meloxicam) might be used to reduce inflammation. During the injection procedure, the smallest suitable needle gauge will be used to minimise tissue damage. The needle will be maintained in position for a few seconds following administration to ensure full delivery of the dose and to minimise leakage of the antigen into the hypodermal layer, which may otherwise contribute to local skin inflammation.

We are following specific regulatory procedures for monoclonal antibodies production. These procedures include submission a request to the University of Cambridge AWERB including:

1. The likely costs to the animals, the expected benefits of the work, and how these considerations balance.

2. Promoting the development and uptake of reduction, refinement, replacement, and other alternatives in animal care and welfare where they exist.

3. Each year, I have to provide Animal Welfare and Ethical Review Body annual report for the production of antibodies using live animals.

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

Our group has an excellent reputation in the field of complement immunology, and we keep ourselves updated with all new and novel experimental designs and models in our field. This includes non-animal models such as the engulfment of bacteria by white blood cells. All newly published data in our field are discussed internally within our group to enrich our knowledge and that helps us to find novel experimental designs or other non-animal models that could help in our study. In addition, all our experiments will be conducted in a way that allows us to publish our results according to the ARRIVE guidelines. we will follow all guidelines provided by LASA, PREPARE and NC3Rs.

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

All efforts will be made to follow the 3Rs throughout the course of this license by learning from previous experience and any new studies we may run. We have signed up for the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) newsletter and will regularly check for the latest updates on their website. In addition, guidance literature from the Laboratory Animal Science Association (LASA) will be considered. Related events such as conferences and symposiums will be attended.

A retrospective assessment of refinement will be due by 28 November 2030

The PPL holder will be required to disclose:

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- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

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