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NON-TECHNICAL SUMMARY

Elucidating the Mechanisms of Plasma Cell and Memory B-Cell Differentiation in the Germinal Centre Reaction

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Immune system, Vaccination, Infectious disease, Autoimmune disease, Blood cancer

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To better understand how B lymphocytes differentiate into high-affinity plasma cells (PCs) and memory B cells during the germinal centre (GC) B-cell reaction of the T cell-dependent immune response in order to develop more effective strategies for vaccination and the targeted inhibition of activated B cells in autoimmune disease and malignant B cells in lymphoid cancers.

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?

B lymphocytes generated in T cell-dependent immune responses are critically involved in several diseases, either through mediating protective functions in infectious disease, through their aberrant activation in autoimmune disease or through oncogenic transformation in blood cancer.

First, the generation of high-affinity antibodies in response to microbial infection and vaccination is fundamental for the organism’s immunological protection against infectious agents. Central to this process is the germinal centre (GC) B-cell reaction of the T cell-dependent immune response. The GC reaction produces long-lived plasma cells (PCs) that secrete highly specific antibodies against the immunizing pathogen, often establishing life-long immunity.[1-5] However, not all pathogens or vaccines induce durable antibody responses, leaving the body susceptible to subsequent infections, a long-known circumstance and more recently highlighted by the SARS-CoV2 pandemic.[6-8] Thus, there is a need to better understand the molecular mechanisms underlying PC differentiation in the GC, because this may inform the development of novel strategies to enhance the humoral immune response to vaccination and increase durability.[6]

Second, several autoimmune diseases are associated with GC-derived PCs that secrete self-reactive antibodies. Thus, insights into how PC development occurs during the GC reaction may provide the mechanistic rationale to inform new treatments that inhibit biological programs activated in pathogenic GC-derived PCs of inflammatory autoimmune conditions such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA).[9-11]

Third, the vast majority of B-cell lymphomas and PC malignancies originate from the malignant transformation of GC B cells or B cells that have undergone the GC reaction. Indeed, cancers of B cells represent a major public health concern in the UK. In 2018, 14,000 new lymphoma cases and an additional 6,000 myeloma cases were diagnosed, with more than 4,900 and 3,100 reported deaths, respectively (numbers from Cancer Research UK). Understanding the molecular mechanisms of GC B-

cell differentiation is a prerequisite to better understand the transforming events leading to lymphomas and PC malignancies, which will provide the basis for developing more specific and effective anti-cancer drugs.[12]

Thus, the new insights into how B cells differentiate into high-affinity PCs and memory B cells upon antigen-activation arising from our proposed work is expected to have a significant impact on either the prevention (infectious disease) or targeted inhibition (autoimmunity, blood cancer) of several diseases. Our goal is to exploit the findings made in mice for rapid translation into public health (vaccination) and the clinics (treatments of autoimmunity and blood cancer).

1. MacLennan Annu Rev Immunol 12:117, 1994 (PMID: 8011279). 2. Victora & Nussenzweig Annu Rev Immunol doi: 10.1146, 2022 (PMID: 35113731). 3. De Silva & Klein Nat Rev Immunol 15:137, 2015 (PMID: 25656706). 4. Nutt et al Nat Rev Immunol 15:160, 2015 (PMID: 25698678). 5. Chang et al Immunol Rev 283:86, 2018 (PMID: 29664564). 6. Amanna & Slifka Immunol Rev 236:125, 2010 (PMID: 20636813). 7. Slifka & Amanna Front Immunol 10:956, 2019 (PMID: 31118935). 8. Kellam & Barclay J Gen Virol 101:791, 2020 (PMID: 32430094). 9. Wahren-Herlenius & Dörner Lancet 382:819, 2013 (PMID: 23993191). 10. Humby et al PLOS Med 6:e1.doi:10.1371, 2009 (PMID: 19143467). 11. Young & Brink Immunol Cell Biol 98:480, 2020 (PMID: 32080878). 12. Basso & Dalla Favera Nat Rev Immunol 15:172, 2015 (PMID: 25712152).

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

Our proposed studies are expected to identify previously unknown genes or pathways that *1)* provide candidates for a targeted modulation of plasma cell (PC)-precursor development in the germinal centre (GC) that may yield PCs with longer durability or in greater numbers, thus enhancing the efficacy of the antibody response to vaccines directed at preventing infectious agents, or *2)* represent Achilles' heels that are suitable targets for a drug-mediated inhibition of pathogenic plasmablasts secreting autoantibodies as well as aggressive GC-derived B-cell lymphomas and PC malignancies. This will guide the development of molecular approaches to improve antibody responses to vaccination on the one hand and therapeutic targeting of genes involved in the pathogenesis of certain autoimmune diseases and blood cancers on the other. As an example for the latter, the results may provide the basis for the development of transcription factor-targeting therapeutics such as small molecule inhibitors and proteasome-targeting approaches (PROTACs), which are projects which could be undertaken in collaboration with structural biologists. Importantly, the identification of 'druggable' targets for anti-cancer therapies may provide the basis for identifying new prognostic and diagnostic markers that allow a more refined stratification of patients with these cancers, and to inform the development of highly specific anti-lymphoma and anti-PC malignancy (e.g. multiple myeloma) drugs that could increase the efficacy of currently used treatment regimens. By focussing on identifying the mechanisms of molecular pathogenesis in autoimmune disease and blood cancer – the prerequisite of precision medicine –, the work will directly contribute to paving the basis for personalized treatment of these diseases. All outcomes of the project will be published in peer-reviewed journals and thus disseminated among the basic and clinical scientists.

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

Understanding the molecular mechanisms of the GC reaction of the humoral immune response in which long-lived PCs and memory B cells are generated is currently a major focus in the field of adaptive immunity, an area which encompasses the study of vaccination strategies as well as autoimmune disease and blood cancer. Thus, there is a large research community working on elucidating the cellular dynamics of the GC reaction, which besides B cells involves several other immune cell types. The quest to understand the basis of the GC reaction in establishing humoral immunity is expected to provide the framework for developing new ideas on how to manipulate the GC response in order to *1)* enhance the antibody response to vaccination and increase durability, and *2)* to control chronic inflammatory autoimmune conditions in which GC-derived PCs secrete pathogenic autoantibodies, and to *3)* identify targetable vulnerabilities in GC-derived blood cancers. Therefore, in the short term, the results from our proposed project on dissecting the molecular mechanisms of the GC reaction and the development of PCs and memory B cells in the GC are directly relevant for academic researchers working in the fields of adaptive immunity, vaccinology and chronic inflammatory/autoimmune disease as well as lymphomas and PC malignancies. In addition, since many molecular mechanisms are shared among immune cell types, this work will also provide a conceptual framework for the functional analysis of other immune cell types involved in human disease, such as in T-lymphocyte subsets, which may be exploited for the development of precision medicine-based therapies.

Equally important, the results emanating from our research may spur collaborations with pharmaceutical companies which have efficient pipelines to screen for and test drugs or biologicals against putative targets, or available drugs could be repurposed for autoimmune or lymphoma therapy.

Our ultimate goal is to improve the health and wellbeing of patients, either by preventing break-through infections due to ineffective vaccination, of by inhibiting disease-causing aberrantly activated or transformed B cells. In the longer term, the insights deriving from our proposed work which through publicizing the results will have spurred translational work by us and other national and international researchers are expected to benefit patients and their carers.

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

The project team including the national and international collaborators which has been assembled to dissect GC B-cell and PC development consists of the leading investigators in their respective fields, thus ensuring that the proposed study aims can be completed in the allocated time. Laboratory expertise will be disseminated through collaborations alongside internal and external seminars, in addition to providing more detailed information on methodologies to interested researchers responding to our publications (see below). We will communicate with our local collaborators through organized, regular meetings, and with our external collaborators through teleconferences throughout the project.

External dissemination of our results to lab-based academics and clinician-scientists will be achieved via peer-reviewed publications and presentations at national and international scientific meetings, including immunology and haematology meetings. Since the immunology community of the UK is well-connected and interactive, as demonstrated by the swift and effective response to the COVID-19 pandemic, there is a real possibility of a rapid clinical translation of findings resulting from basic science. The same applies to the UK lymphoma research community, with which our team is tightly connected. Overall, our research will benefit external researchers at the national and international

levels, which importantly may spur additional research into the subject area using specific expertise provided by the respective research groups.

Long-term, there is potential to translate our findings on novel potential targets for pharmacological inhibition into clinical trials, since several investigators connected to us who lead, or are involved in running national clinical trials for rheumatoid disease and B-cell lymphomas/plasma cell malignancies, are interested in the development of improved treatments.

For non-academic users, we communicate our research to the interested lay audience and the charities through public/patient partnership engagement activities, laboratory tours and scientific presentations, as previously realized by the lead investigator and co-investigators. We recognise that vaccine development for infectious diseases is now in the public eye. New insights into the molecular mechanisms of long-lived PC development that may uncover novel ways of manipulating the antibody response will potentially provide new avenues for vaccine development and precision medicine-based treatment strategies in autoimmune conditions and blood cancer. Such insights are expected to lead to media coverage and dissemination to the public. Our published work will be transmitted to other media and the public through the local Press Office. In case our findings warrant wider media coverage, we will work with the media and guarantee availability of our team of investigators. Moreover, the annual public engagement events of Festival of Science organized at local or national levels provide a platform to communicate our results to schools and the public.

Species and numbers of animals expected to be used

- Mice: 2000

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 plan to employ transgenic mouse models in order to better understand the molecular mechanisms that establish an effective humoral immune response on the one hand and that can lead to the aberrant, disease-causing activation of B cells in autoimmunity and blood cancer on the other.

The mouse is the model organism of choice to study the biologically highly complex vertebrates by directed genetic manipulation, and the generation and use of transgenic mice to analyse the function of genes associated with human disease has over the last decades led to tremendous insight into the precise mechanisms of disease development and has formed the basis for a great number of clinical applications in humans. Of exceptional importance for the planned studies is the fact that the immune system of mice is well characterized and closely resembles that of the human, which has the important implication that insights from mouse models can be directly translated to humans. Indeed, the mouse and the human genome are the most highly homologous genomes of the large vertebrates. Moreover, a wealth of commercial reagents and techniques for analysing the lymphatic system of mice are

available. For these reasons, this species is most appropriate for an analysis of body cells in health and disease.

Specifically, the transgenic mouse models described in this project are required for effectively recapitulating the human germinal centre (GC) B-cell response of the T cell-dependent immune response, which allows the focused study of the biological role of particular genes in the GC B-cell response.

With regards to the choice of life stages: a precondition for the planned studies is a functional immune system as it is present in adult humans. In mice, a functional immune system is established past six weeks of age. We plan to immunize mice when they are 8 to 12 weeks old.

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

Since the experiments are performed with genetically modified animals (GMAs), tissue biopsies will be taken by ear punch and subjected to molecular genotypic analysis to determine the genetic status.

Mice will be administered substances by intraperitoneal (most cases) and intravenous (rare cases) injection and/or in drinking water. Administered substances include T cell-dependent antigens (sheep-red blood cells and nitrophenyl-Keyhole Limpet hemocyanin) with or without adjuvants (Complete or Incomplete Freund's Adjuvant) and substances that label proliferating cells (e.g. BrdU, EdU). For most experiments, one animal may receive a maximum of 2 different substances in a maximum of 3 administrations repeated 2 to 42 days after initial immunization.

Blood (50µl) may be taken from a superficial vessel, e.g. tail vain 7 days before the first immunization and with 7-42 days interval after the first immunization and then throughout the experiment. The purpose of the blood withdrawal is to determine the titer of model antigen-specific antibodies in the serum that are generated during the T cell-dependent immune response.

Typical experiment A: a mouse is immunized with a T cell-dependent antigen and then humanely killed for analysis of the lymphoid organs 10 to 14 days later.

Typical experiment B: a mouse is immunized with a T cell-dependent antigen and then 21 days later the immunization is repeated, before it is humanely killed for analysis of the lymphoid organs 10 to 14 days later.

Blood may be taken in A and B 7 days before the first immunization and 7 to 14 days after the first immunization.

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

Immunizations with the T cell-dependent antigens sheep red blood cells (SRBC) and nitrophenyl-Keyhole Limpet hemocyanin (NP-KLH) in Freund's Adjuvant are at the centre of this project. In a low percentage of mice, expected adverse effects are mild inflammation with oedema and erythema (granulomatous peritonitis) at the site of SRBC and antigen-in-adjuvant administration. Freund's Adjuvant is chosen as adjuvant because in combination with the NP-KLH model antigen it gives the best antigenic response as measured by germinal centre (GC) induction. Moreover, by using Freund's

Adjuvant, repeatability of previous work is ensured, since in the past this adjuvant has been used in a large number of studies, including ours. This will allow to assess and compare our results from the proposed immunization experiments on the background of published studies.

Mice displaying an exaggerated response to the induced inflammation will be monitored daily by the animal technican and advice sought from the NVS when erythema and pustule formation is associated with lethargy due to hyperthermia; where necessary mice will be removed from the study and culled promptly by a humane killing method. Furthermore, the body condition of mice will be monitored and any mice culled if they show signs of ill health such as piloerection / hunched posture / lack of appetite for a period of 48h.

No adverse effects are expected to be associated with blood sampling or administration of BrdU or EdU.

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)?

Protocol 2 falls under the category moderate severity since a fraction of mice may develop mild inflammation with oedema and erythema at the site of antigen administration. The percentage of immunized animals displaying an exaggerated response to the induced inflammation is estimated to be lower than 5%.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of this project is to understand complex biological processes, most notably including the molecular mechanisms of the T cell-dependent antibody response which generates high-affinity plasma cells (PCs) and memory B cells, in primary cells in vivo. This information is expected to be directly relevant to the development of vaccination-enhancing compounds as well as novel anti-inflammatory and anti-blood cancer therapies. Regarding this particular project it is important to note that the development of PCs and memory B cells occurs in the germinal centre (GC), which is a specialized microenvironment which develops in T cell-dependent immune responses upon antigen administration in the spleen and lymph nodes. The GC B-cell response involves complex molecular processes that contribute to the affinity maturation of the immune response as well as to the generation of immunological memory; most important to note here is the process of somatic hypermutation of the

antibody genes, which is a DNA-modifying mechanisms (and thus a potentially mutagenic process occasionally giving rise to B-cell cancers) that together with selection mechanisms generates antibodies with improved binding to the disease-causing pathogens.

So far, it has been impossible to reproduce the GC microenvironment in vitro, which is due to the fragile nature of these cells that do not survive in culture (as documented by extensive literature), as well as to the complex composition of this microenvironment, which includes various amounts of B cells, T cells, follicular dendritic cells and macrophages that require the activity of multiple cytokines and chemokines for their function and survival. None of these complex individual events occurring in the living organism over a large time-window are available via other cellular systems.

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

For the gene/pathway identification studies, we have considered cell culture systems that co-culture human tonsillar GC B cells on stromal cells (organoids), which are being developed with the aim to mimic the GC reaction occurring in vivo.

For some aspects of our study regarding the gene/pathway validation studies as well as e.g. the investigation of the deregulated function of proto-oncogenes, whenever possible we plan to investigate those at the molecular level using appropriate in vitro cultures of human cell lines.

Why were they not suitable?

Until now, no functional in vitro cell culture system has been developed that faithfully mimics all aspects of the in vivo GC reaction. A major caveat is that the in vitro cultured cells do not undergo somatic hypermutation, and the requirements for selection of high-affinity B cells that include a specific subset of T cells and follicular dendritic cells are as of yet impossible to mimic in the culture. Thus, in the absence of an in vitro system for the highly complex process of GC B-cell development, the use of animal models remains the only rational approach to study their role in the context of the complex living organism.

Another limiting factor of using human B cells is that in our proposed project, we aim at the identification of very small GC B-cell subpopulations that become visible and thus identifiable only through the expression of a living-color fluorescent marker protein which has been targeted into the mouse genome so that it is expressed in the GC B cells of interest. Indeed, a major aim of the project is to unambiguously identify and characterize these functionally defined GC B-cell subsets in mice by benefitting from the possibility of targeting the mouse germline, which will provide information to identify the corresponding cell subset among human GC B cells.

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 previous experience documented by relevant publications [see Experience section below] allows us to ensure that animal usage is kept to a minimum with experiments designed such that we expect the outputs to be measurable and reproducible.

In order to achieve statistical significance and experimental reproducibility, we use 5-10 mice per genotype group. For the experiments involving immunization, 5 mice per experimental point is the commonly used number to obtain interpretable, statistically significant results, as extensively documented in the scientific literature. For example, in the experiments utilizing flow cytometry, measurement of newly identified markers, assuming a 20% change in samples between two genotypes, with an SD of 10%, gives 5 mice per group for an alpha level of 0.05 with 90% power when using a 2-sided Student's t-test. This calculation thus represent the optimum number of animals needed to attain statistical significance of $p<0.05$ with a 90% probability. Because of the nature of the immunization experiments, outlayers can occur e.g. due to inefficient immunization (documented by studies), thus requiring sample sizes of more than 5 mice, with the actual number depending on the outcome (phenotypic difference among the genotypes). For functional studies on B cells, due to the limited number of B cells one can obtain from a mouse, appr. 10 mice per group are required for functional in vitro studies (e.g. assays for activation of biochemical signalling pathways and cell differentiation) on isolated B cells aimed at understanding the molecular consequences of the B cell-specific deletion of the signalling molecules and transcription factors.

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

The numbers of mice to be maintained and used in experiments will be kept at a minimum to ensure that reliable experimental data is obtained (see power-calculation example in Figure 2 of Protocol 2 in the section "How will you determine group sizes"). To achieve this, we based our group size determination and randomization on the vast literature of immunological research with animal models, in addition to implementing the Experimental Design Assistant (EDA) from NC3Rs in our study design. Moreover, we follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines to ascertain that our research design is compatible with the ARRIVE checklist of recommendations. A potential sex bias in the resulting data is eliminated by using both male and female mice.

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

Our project includes efficient breeding to reduce the number of GMAs that are not of the desired genotype, which is a general caveat of the interbreeding of multiple alleles. For example, in the experiments with the experimental cohort of *geneX*$^{fl/fl}$*geneY*-Cre (tissue specific knockout), *geneX*$^{fl/+}$*geneY*-Cre and *geneX*$^{+/+}$*geneY*-Cre, we follow a breeding strategy in which *geneX*$^{fl/fl}$ mice are intercrossed with *geneX*$^{fl/+}$*geneY*-Cre mice to obtain Cre-expressing homozygous and heterozygous mice for the floxed allele, and *geneX*$^{+/+}$*geneY*-Cre with *geneX*$^{+/+}$ mice to obtain the Cre-expressing control mice. This strategy, which is possible to follow since all mouse lines are on the C57BL/6 genetic background, considerably reduces the generation of ‘unwanted’ genotypes that would have to be humanely culled.

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.

As a general consideration which is highly applicable to refinement: the mouse is the model organism of choice to study the biologically highly complex vertebrates by directed genetic manipulation, and the generation and use of transgenic mice to analyze the function of genes associated with human disease has over the last decades led to immense insight into the pathogenetic mechanisms of disease development and has formed the basis for a sizable number of clinical applications in humans. The endogenous mouse genome can be manipulated in mouse embryonic stem (ES) cells, which limits the use of animals in this phase (i.e. by not having to identify and select naturally occurring mutants).

The project entails minimal, momentary pain and distress associated with genotype identification, intraperitoneal (i.p.) and intravenous (i.v.) injections, blood withdrawal, and euthanasia. Immunization by i.p. injection may occasionally cause localised inflammation with oedema and erythema (granulomatous peritonitis), which however does not necessarily lead to chronic distress. Mice will be monitored daily for general health status following i.p. immunization.

The severity limit for most mice will be mild. Specifically, the majority of animals produced under the breeding program will be used to supply tissues for in vitro and lymphoid tissue analyses (flow cytometry, immunohistochemistry). The mouse lines used in the project have no defects beyond alterations in or loss of immune cell populations and suffer no ill effects in an SPF animal facility. The low percentage of mice displaying an exaggerated response to the induced inflammation due to immunization with the T cell-dependent antigens sheep red blood cells (SRBC) and nitrophenyl-Keyhole Limpet hemocyanin (NP-KLH) in Freund’s Adjuvant (moderate severity) will be monitored daily for signs of ill health such as piloerection / hunched posture / lack of appetite and advice sought from the NVS; where necessary mice will be removed from the study and culled promptly by a humane killing method. Immunizations with SRBC and model antigens such as NP-KLH in adjuvant are currently the most refined methods to study the T cell-dependent immune response in mice. These immunization models are well established and are required for immunological research, and in addition allow the comparison of results from immunization experiments among different research groups. No adverse effects are expected to be associated with blood sampling or administration of reagents to determine proliferation (e.g. BrdU).

For all procedures, we will apply the least invasive methods of dosing and sampling appropriate to the objectives of the experiment, including the use of anaesthesia for humane restraint where necessary.

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

With regards to the life stage: a precondition for the planned studies is a functional immune system as it is present in adult humans. In mice, a functional immune system is established only past six weeks of age. Thus, by studying the immune system of adult mice, we ensure that our findings are relevant to adult humans.

The adaptive immune system as found in humans, a complex system of cells and organs that recognize, destroy and memorize invading pathogens, has evolved only in higher vertebrates. Of note, the mouse and the human genome are the most highly homologous genomes of the large vertebrates, and the immune system of mice is well characterized and closely resembles that of the human, with both being different from other species as e.g. fish and birds (chicken).

With regards to animals that have been terminally anaesthetized: this option is not applicable as the project does not include the highest severity level.

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

In our facility, animals are monitored daily and their health status is recorded using an established animal welfare scoring system. After i.p. injection of substances, animals are closely observed for adverse effects, and monitored daily for signs of ill health such as piloerection / hunched posture / lack of appetite.

We have established a protocol for the use of Freund’s adjuvant but will also actively seek to replace this adjuvant with less invasive substances for the emulsification of antigens, should these become available during the project, by conducting small scale pilot studies and comparing results with our established protocol.

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

We follow the most recent Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, which are currently The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published in PLoS Biol 18:e3000410, 2020; PMID: 32663219; PMCID: PMC7360023.

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

We will make use of the NC3R website and available 3R online resources. Through our connection with other local and national research groups, we have the opportunity to discuss protocol-relevant issues that may benefit our protocols.

We will closely follow any new developments in the area of cell culture systems that co-culture human tonsillar GC B cells on stromal cells (organoids), as advances may allow to study some aspects that we currently plan to investigate in animals by implementing organoids. To this end, we will follow newly-published research (including Bioarchive or Medarchive servers) and screen abstracts of national and international conferences.

We participate in events organized by the local Animal Welfare Ethical Review Body (AWERB), which also informs us promptly about any new developments in the field.