Home Office

NON-TECHNICAL SUMMARY

Papillomavirus Transmission, Infection and Lesion Formation

Project duration

5 years 0 months

Project purpose

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

Key words

Papillomaviruses, Cancer, Infectious Disease, Virus Transmission, Viral Immunity, Viral Immunity

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Rabbits	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 establish how papillomaviruses are transmitted, how lesions form and are maintained, and to find new ways to prevent transmission and treat and treat clinical disease .

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?

Papillomaviruses (PVs) have been discovered in a wide array of animals. More than 300 PVs have been identified, including over 200 human papillomavirus (HPV) types. HPVs infect humans and cause epithelial lesions with different clinical outcomes, ranging from non-cancerous hyper-proliferative lesions such as warts, to subclinical (not apparent) lesions that can in some instances progress to high-grade neoplasia and cancer. HPV infection is responsible for approximately 6.1% of all human cancers including cervical, head and neck, anal and conjunctival cancers, however 90% of HPV infections are cleared by the host immune response. HPV vaccines have been proven to be effective and are expected to make great impacts on HPV-related disease, but have some limitations:

a) They are only effective for individuals who have never been infected with vaccine type HPVs (i.e. they are prophylactic rather than therapeutic).

b) Because of their cost, these vaccines are not widely available in developing countries where resources are limited.

c) Vaccine hesitancy is restricting implementation even in developed countries with vaccine programs.

Therefore, there is ongoing need for the development of antiviral agents to treat existing HPV infections, and an efficient strategy for the management and control of HPV infections through screening, including cervical screening, which is currently being modified to incorporate HPV DNA testing .

As HPVs are host-specific and cannot infect other animals, and there is no precise animal model for HPV infection. However, the general strategy of all PVs to accomplish their various life cycle stages in the skin are similar, with different papillomaviruses having similar molecular functions for their gene products. Although many aspects of the infection route are not fully worked out i) PVs target the skin, which consists of multiple cell layers; ii) after PVs have infected cells in the lower (basal) layer of the skin, infected cells are maintained for years to decades, modulating proliferation and commitment to differentiation of the skin cells; iii) Once the infected cells leave the basal skin layer, the productive life cycle is triggered and new viruses are produced; iv) PV is shed inside dead cells from the surface of

infected skin, and transmitted via direct or indirect contact; v) PV infection is controlled by the host immune system, which modulates viral gene expression.

Given this common strategy of PV infection, we aim to use animal models to elucidate how PVs are transmitted, and how new lesions form and are maintained, in order to develop new insight into PV transmission mechanisms, and the development of anti-papillomavirus agents/treatments and infection control strategies. These aims can be achieved using models that allow us to investigate all aspects of the virus life cycle (transmission, infection, lesion formation, and the production of new virus) in the presence of an intact host immune system.

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

During this project, we aim to better understand:

1. How mouse PV is transmitted and infects new sites, particularly how virus preparation (purified virus, cell-free virus, and virus in exfoliated dead cells (squames)) affects virus survival and infectivity. We will evaluate the efficacy of disinfectants currently used in clinical settings on virus infectivity. Together with a parallel analysis using human PV in tissue culture models, we will examine how papillomavirus transmission can be controlled and prevented. We expect to achieve this goal within 3 years.

2. The early stages of lesion formation following infection. We want to define the molecular pathways that are modulated by the virus to maintain infection in the basal (lower) layer of skin cells, and how virus genes do this (achievable in 3 years). We also want to develop ways of manipulating these pathways to inhibit the maintenance of infection in the basal keratinocyte (skin cell) layer, leading to the development of anti-papillomavirus therapy/agents. This will depend on the progress of a parallel study using keratinocyte tissue culture models to investigate the function of virus protein function on keratinocyte proliferation and differentiation. However, we expect to have a rudimentary understanding of this within the 5-year period.

3. How mouse PV gene expression patterns in mice vary at the different body sites in mice (achievable in 3 years). Our longer-term aim is to understand how virus gene expression patterns contribute to the difference in the behaviour of the infected lesion (phenotype), and to relate such differences to expression seen in clinical samples infected by HPV. We expect to have a basic understanding of this within the 5-year period.

4. The molecular process that drives lesion regression. This is a more substantial goal than those described above, but during these 5 years we aim to look at the lymphocytes (small white blood cells) and the host's immune response in the lesions that regress.

We estimate that four major publications will come from this proposed work, the first relating to ‘Papillomavirus transmission, lesion formation and disinfection’, a second relating to ‘The molecular mechanisms of early stage papillomavirus lesion formation’, a third relating to 'The process of lesion regression of papillomavirus infection; the implications of the host immune system', and a fourth relating to 'Tissue culture models of the cervical transformational zone, and the dysregulation of viral gene'.

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

The results will benefit the scientific community, by providing a comprehensive understanding of how PVs are transmitted and form lesions, and how infection is controlled by host immunity. The impact of outputs from the first objective (viral transmission) will provide a new insight into how HPV transmission can be controlled and prevented, which will directly affect clinical practice. In the longer term, the outputs from the second and third objectives will provide identify key cellular pathways that PVs regulate to form and maintain lesions at specific sites of infection. Those interested in the development of anti-HPV treatments/agents will also benefit from this work.

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

The scientific results will be disseminated globally primarily through publications as well as preparation of review articles, book chapters, and lectures at scientific and other meetings.

Species and numbers of animals expected to be used

- Mice: 3710
- Rabbits: 96

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.

There is no experimental model to investigate all stages of PV infection (infection, lesion formation, virus production, and virus regression) without the use of animal models. Only animal models will allow us to investigate all aspects of these stages. Each PV is highly host-specific and can infect only their natural host. However, all PVs are considered to share fundamental strategies in their life cycle. Thus, we can understand how the human PV transmits, infects and forms its lesion by investigating animal PVs. Using the mouse PV model, we can also investigate virus transmission and lesion formation using various genetically modified mouse strains. With the rabbit PV model, we can specifically investigate how the host immune system responds to PV infection and resolves it.

Some PVs, including HPV, cannot be propagated using a tissue culture model. Transplanting infected cells into mice is currently the only way that we can propagate infectious PVs for use in other experiments.

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

Small areas of skin will be gently scarified before infectious material is applied. A wart-like lesion will grow at the inoculated site and the development, and in some cases regression, of the lesion will be monitored over a period of up to 6 months.

After virus infection, animals will receive reagents or treatment (e.g. cryotherapy) that may affect the process of lesion formation/persistence. The number of procedures that mice and rabbits will be subject to will be kept to a minimum to limit discomfort, while allowing the generation of scientifically useful data. A set of procedures will be repeated no more than three times per animal, with intervals between treatments that allow for full healing (e.g. following mechanical abrasion). Ten percent of animals may receive both reagents and treatment, the rest will receive one of the two, or neither. Animals will be continually monitored for adverse effects post-treatment, and if necessary, the intervals between treatments will be adjusted or the treatment will be suspended. Similarly, rabbits will be inspected regularly and, in some cases, biopsies (up to three) and blood samples (up to two) may be collected.

For infectious papillomavirus production, tissues/cells will be transplanted into mice under the coating covering each kidney by surgical procedure. Transplanted tissue/cells will be collected after three months, after the animals have been killed.

Genetically modified mouse strains which are not commercially available will be maintained/bred under this project, and procedures required for this will be carried out. None of the genetically altered mice are expected to develop any adverse effects.

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

Proliferative lesions will develop at the site of infection (warts). In mice, the lesions are self-limiting and localised, and will not affect the general health and welfare of the animals. Animals will be killed by a humane method before they show moderate clinical signs, such as large lesions or secondary lesions that interfere with the normal processes of feeding and movement within the cage. In rabbits, the lesion naturally disappears within 4 months. In mice, the transplanted tissue/cells will not cause any clinical signs.

Animals will be closely monitored during and after each procedure, and the animal will be treated if adverse clinical signs (such as weight loss, lack of normal movement or other abnormal behaviour) are seen. If there is no response to treatment, the animal will be killed by a humane method. Bodyweight will be recorded when the experiment starts and will be monitored regularly and compared with age and ‘free feeding' matched control animalscontrol animals.

Although we expect some transient soreness at the scarification site immediately after infection, the non-cancerous lesions that develop as a result of infection are not expected to be painful for the animal. Painkillers will be provided if required.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority of mice (95.4%) will experience procedures of only mild severity, with the remaining mice (4.6%) undergoing procedures of moderate severity. Only mild severity procedures will be used with Rabbits.

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

- Kept alive
- 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 can investigate all the stages of PV infection from transmission/infection, lesion formation, and lesion regression, to reactivation at particular epithelial sites, only by the use of animal models. Importantly, we can only investigate the role of the immune system in PV lesion regression using the rabbit PV system. By using these animal models we can produce a more complete picture of PV/host interactionsinteractions.

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

We already make extensive use of tissue culture and organotypic raft culture (which allows the formation of skin tissues in the lab), to study the papillomavirus life cycle. We also employ a culture system to study infection and lesion formation in patient biopsy material. Additionally, we compare the results from animal experiments with clinical observation of patient material. Such comparative analysis allows us to develop our understanding of how papillomaviruses interacts with skin (epithelial) cells.

Why were they not suitable?

The raft model and analysis of clinical specimens are useful; however, we need to use animal models to address particular questions concerning early lesion formation, regression and the associated immune responses. In addition, some human papillomaviruses can only currently be grown outside of their human host, in animal models.

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?

Production and maintenance of genetically altered mouse colonies (2 strains concurrently) is estimated to require 2000 mice over 5 years. The standard protocols for genetically altered mice (protocols 1 - 5)

may require 410 mice over 5 years, as we expect to need two strains to make the embryo stock and/or to re-establish the strain from the sperm or embryo stock.

The PV infection protocol (protocol 6) is estimated to require a maximum of 1000 mice (24 mice per experiment, for 50 experiments) over 5 years. The production of 'infectious PV protocol' (protocol 8) is estimated to require 100 mice (4 mice per PV stock, for 25 PV types) over 5 years.

The rabbit PV infection protocol (protocol 7) is estimated to require 100 rabbits (8 rabbits per experiment, for 12 experiments) over 5 years.

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

Our experiments will be exploratory, and primarily observational or qualitative. They will address different features of disease pathology and viral gene expression, such as how the host immune system controls infection, how PV changes the behaviour of infected cells to form lesions, as well as how the site of infection affects PV behaviour.

Substances which are expected to modulate virus function are commercially available, and routes of administration, dosage volumes, frequencies and durations will be based on established protocols from published literature. We will use the NC3Rs experimental design assistant tool and the PREPARE guidelines, and submit a protocol amendment along with justification if our analytical requirements change change in future.

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

We are already involved in the analysis of virus expression and virus protein function in clinical specimens and skin (epithelial) tissue culture models, and we are using these models to develop our hypothesis of how PVs interact with the skin cells (keratinocytes) that they infect. Before conducting any animal experiments, we will make extensive use of tissue culture and organotypic raft culture (a system that permits 3D modelling of virus infection in lab recreated skin tissues), to study the biology of PVs in vitro. Experiments which specifically require the use of animal models will be conducted once this has been done.

We will keep animal strains that are not commercially available as embryo stocks, and will breed and maintain living colonies only when necessary during the project in order in order to minimise the use of animals.

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.

Previously, the most appropriate animal model for the study of PV infections was the rabbit, and we have used the cottontail rabbit (CRPV) and rabbit oral (ROPV) papillomavirus systems in previous studies. These models are difficult to use, and we have found that rabbits may suffer weight loss upon treatment with immunosuppressant drugs. The identification of a mouse PV has taken time to achieve, but recently one has been described in the literature and is reported to produce typical papillomas (warts) in genetically modified immunodeficient mice. As a result, we can now assign the majority of our studies to the mouse PV model. However, the rabbit PV models are still useful, and essential when studying how the host immune system responds to PV during the natural course of infection. Other papillomavirus models are in larger animals (cows, dogs) or unconventional laboratory animals (e.g. the multimammate rat).

Papillomavirus lesion formation requires scarification of the skin to allow access of the virus to the lower (basal) epithelial layers. Scarification is minimised to limit bleeding and is performed under anaesthetic. The process of lesion formation causes no pain to the animal, and will routinely be carried out on the tail.

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

Each PV can only infect and form lesions in its natural host, and as a result we need to use mice to study the mouse PV, and rabbits for the rabbit PV. We are using animals of the lowest sentience that are susceptible to PV infection.

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

Working closely with the biomedical services staff, we will monitor animals on a daily basis. Animals must be allowed to acclimatise to new surroundings and personnel before any procedures are performed, to minimise stress and safeguard animal welfare and research results. Personnel will be trained to handle mice and rabbits correctly. Especially during and after procedures, animals will be monitored closely for adverse clinical signs, and the experiment/procedure will be terminated if necessary, to stop suffering. It is expected that animals will endure some discomfort associated with the surgical procedure. This will be minimised by use of good surgical technique, and pre-emptive and post-operative analgesia will be administered.

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

We will follow the Laboratory Animal Science Association (LASA), PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines.

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

We subscribe to the Tech3Rs newsletter published by NC3Rs, and will keep up-to-date with our department's circulated 3Rs publications and website.