GM Microorganism Risk Assessment

Full Assessment, New Format

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Final Classification of Project: **Class 2**

Title: **Evaluating in vivo immune responses to defined antigens**

Lab: Floor 2
Bldg: Wellcome Trust Biocentre

1: Brief Description of Project

Recombinant Vaccinia virus will be used to evaluate the in vivo immune responses to defined antigens engineered into the virus. This work involves both the propagation of virus in vitro as well as the infection of mice and harvesting of tissues for analysis.

The recombinant vaccinia viruses to be used have already been constructed by a collaborator through homologous recombination of plasmid DNA (pSC11) into the thymidine Kinase gene of vaccinia virus (western reserve, WR). Inserted genes encode antigenic determinants such as; mouse melanocyte antigen; influenza antigens; antigens from lymphocytic choriomeningitis virus (LCMV) and non-functional murine proteins.

2: Hazards to Human Health

(a) Associated with recipient micro-organism

Vaccinia virus has been administered to humans as a means of inoculation against smallpox since the 1800s. It causes acute infections from which a normal, healthy person will recover with life long immunity. Primary vaccination results in a pus filled blister at the vaccination site which scabs and detaches after 14-21 days leaving a typical scar. Conjunctivitis may occur after inoculation to the eye but permanent eye damage is highly unlikely. Infection spreads via contact with the pustular lesions. Further complications are rare in healthy individuals but severe adverse reactions can occur at a rate of approximately 1 per 50,000 vaccinations. Those at high risk include pregnant women, immunodeficient individuals, persons who have suffered from eczema or other acute chronic skin conditions, and persons with life-threatening allergies to certain antibiotics. Due to the potential to cause severe adverse reactions in these risk groups, vaccinia virus is assigned to ACDP Hazard Group 2.

(b) Arising directly from the inserted genetic material (toxin, oncogene)

The antigenic determinants expressed by these recombinant viruses code for non-functional proteins and protein fragments. They are not inherently harmful. They have been inserted using the vector pSC11, a puc derivative, containing an amp resistance gene, beta-galactosidase and the vaccinia 7.5K promoter. They have been designed to contain immunodominant epitopes recognised by the immune system and therefore are more likely to be targeted by the host's immune system.

(c) Arising indirectly from the inserted genetic material (eg alteration of pathogenicity, host range, tissue tropism, mode of transmission or host range)

Insertion of antigenic determinants does not alter the tissue tropism or host range. Nor does it increase the infectivity or pathogenicity of the recipient vector. Antigenic determinants are inserted into the thymidine kinase region of the

vector rendering the recombinant virus attenuated compared to the wild type. The inserted genes pose no risk. Indeed insertion of these genes into vaccinia virus WR reduces the virulence of the virus 10,000 X in mice (Buller et al. 1985 Nature 317:813). However the recombinant virus also retains its replication competency prohibiting any down-grading to Hazard Group 1. Based upon there being no likelihood of any effect on the phenotypic characteristics of the recipient and the non-harmful nature of the insert, the resulting recombinant vaccinia virus vector can be considered equivalent to the recipient vector in terms of hazard status i.e. Hazard Group 2.

(d) Arising from transfer of genetic material to a related micro-organism

Recombination of the modified virus with a wild type could only occur if a host cell was simultaneously infected with the modified vaccinia virus and a wildtype orthopoxvirus. The likelihood of recombination in vivo with wild type virus is extremely low because the only naturally occurring related orthopoxvirus, the monkeypox virus, is confined to Zaire, rendering the possibility of recombination highly unlikely.

3: Assign a provisional Containment Level

○ Class 1/Level 1 Containment ◉ Class 2/Level 2 Containment ○ Class 3/Level 3 Containment

4: Hazards to the Environment

(a) Associated with recipient micro-organism

Vaccinia virus is heat-stable and persists for prolonged periods under normal environmental conditions. Lyophilised, it can maintain potency for 18 months at 4-6 degrees C and is stable when dried onto environmental surfaces. Vaccinia virus has a very broad host range, infecting humans, cattle, sheep, horses, swine, goats, mice and monkeys. However, even after the vaccination of millions of people during the smallpox eradication program, Vaccinia did not establish itself in the environment. A detailed risk assessment carried out on a vaccinia virus recombinant vaccine for rinderpest (see http://www.nbiap.vt.edu/brarg/brasym96/yilma96.htm) demonstrated attenuation in single and double recombinants and no transmission to non-target species even when housed with the vaccinee animals. The assessment references further evidence on the inability of recombinant vaccinia viruses to spread to contact groups. If escape into the environment did occur infected animals would exhibit symptoms similar to humans - pustular lesions at the inoculation site. Recombination of the modified virus with a wild type could only occur if a host cell was simultaneously infected with the modified vaccinia virus and a wildtype orthopoxvirus. As noted in section 2d, the only naturally occurring orthopoxvirus is the monkeypox virus, restricted to Zaire, rendering the possibility of recombination highly unlikely.

(b) Arising from genetic material

In the very unlikely event of a release from the Containment Level 2 Facility, the antigenic determinants inserted into vaccinia pose no hazard to the external environment.

5: Nature of Work

(a) Brief description of nature of work (include maximum culture volumes)

Recombinant vaccinia viruses containing antigenic determinants will either be amplified/detected by infection of a thymidine kinase deficient cell line in vitro, or be used to infect mice.

Propagation of the virus will be carried out in a Containment Level 2 tissue culture suite. Infected cells will be incubated for up to 72 hours allowing the virus to propogate to a high titre. After incubation cells will be collected and lysed to generate stocks of vaccinia at a high titre for murine infection or fixed and stained for a plaque assay.

Murine infection will be carried out in a Containment Level 2 facility in the animal unit. Mice will be infected with a

(b) Is a microbiological safety cabinet or isolator required to protect against aerosol transmission?

◉ Yes ○ No

(c) Waste Disposal

Vaccinia is sensitive to moist heat (121° C for at least 20 min).
Virkon is effective against Vaccinia virus at 1% for 10 min (efficacy data available on manufacturer's web site) and will be used for surface disinfection.

Liquid waste is collected in a sealable, robust, autoclavable container, autoclaved then disposed of to drains. If using a microbiological safety cabinet store the container within the cabinet during use and seal before removal. Aspirator

set-ups are not used.

Solid waste (including plastic pipettes and agar plates) is collected in a lined, biohazard labelled, autoclavable bin, autoclaved then disposed of as normal refuse.

Large glass pipettes are not used.

Sharps waste is collected in an autoclavable sharp-safe container, autoclaved then disposed of as clinical waste. If using a microbiological safety cabinet store the container within the cabinet during use and engage temporary closure before removal. The use of sharps must be avoided unless essential.

(d) Are sharps required? YES or NO - if yes justify use.

YES - for injection of mice. No alternative.
For other activities - No.

(e) If the work involves experimental infection of animals is it known if the animal will shed the genetically modified micro-organism? If YES give details and measures to prevent exposure.

YES – can be shed to the bedding of animals, although as described above, uninfected animals housed within the same cage are at extremely low risk of infection. The risk of infection to humans is minimal as and is further reduced by: wearing appropriate PPE; housing animals in sealed, individually ventilated cages; adhering to strict disinfection and cleaning regimes; use of a Class II microbiological safety cabinet. Cage waste is removed within a Class II cage-change cabinet, double bagged then autoclaved before disposal. Used cages are treated with Sanosil Super 25 (efficacy data available on manufacturer's web site) prior to regular cage washing procedure. Carcasses are double bagged and autoclaved prior to disposal.

(f) If the work involves the experimental infection of plants what is known about the likely route of transmission of the genetically modified micro-organism?

N/A

(g) Where will the genetically modified micro-organisms be stored?

WTB Floor 2 TC suite, -80 freezer in central equipment room and liquid nitrogen cell freezer in central equipment room. Samples in liquid nitrogen cryo-stores will be contained in proper cryo-tubes & stored in the liquid nitrogen vapour phase to eliminate risk of tube explosion upon initial warming. Samples in fridges/freezers will be doubly contained. Fridges, freezers & cryogenic storage vessels will be secure, biohazard labelled & subject to a well maintained inventory system.

(h) How will the genetically modified micro-organisms be transported within/between buildings to minimise risk of spillage/escape?

Samples will be doubly contained during transport & clearly labelled with a contact name & number, the nature of the sample & the biohazard symbol. Inner container/tube will be robust & leak-proof. Outer container will be robust, leakproof & contain enough absorbent material to absorb the total volume of sample should the inner container leak.

(i) Will staff and students receive any vaccinations or health surveillance?

Currently ACDP/ACGM do not recommend vaccination for individuals doing routine work with vaccinia virus although, immunocompromised and pregnant individuals will not work with vaccinia due to the risks to their health should accidental infection occur. If the work is large scale, involves recombinants that are potentially more virulent than the wild type or if the worker requests vaccination, an assessment must be made as to whether it is a appropriate in any given case. None of these apply to this particular situation. See "Vacciniation of Laboratory Workers Handling Vaccinia and Related Poxviruses for Humans" (1990 ACDP and ACGM, HMSO ISBN 011885450X) for further information.

However, all those who may be exposed to the virus must be familiar with the signs of vaccinia virus infection, check their skin frequently for unexplained lesions and report any concerns to CLS Health & Safety immediately. Photographs of VV infections are available by searching for 'smallpox' at http://phil.cdc.gov/phil/quicksearch.asp. Photographs of vaccinia virus infections can also be found in 'Vaccinia (Smallpox) Vaccine Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001', available at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm.

(j) Emergency Plan

Not required - small scale activity and low risk.

(k) Monitoring

Autoclave Testing and Maintenance

During the first four years after installation an annual 12-point validation test, employing independent thermocouples, is used to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves are serviced every 6 months by a reputable service provider and calibrated annually to ensure the validation criteria are met. During normal, daily operation indicator tape and, in the case of liquid waste, a temperature probe placed at the centre of the load, are used to ensure the required conditions are achieved. Servicing and testing is arranged and test reports are kept by the CLS Health & Safety Coordinator.

Maintaining PPE

Users are required to routinely check their PPE (e.g. lab coat, safety glasses) and keep it in good order. Defective PPE must be repaired or replaced immediately. Laboratory Managers are required to ensure the appropriate PPE is readily available and keep an inspection record for non-standard PPE, e.g. that used in Liquid Nitrogen facilities.

Inspections, Audits and Continual Monitoring

Safety Inspections are carried out regularly to ensure health & safety policy & procedures are being followed and that the required risk assessments and training records are complete and up to date. Inspections are timetabled and inspection teams selected by the CLS Health & Safety Working Group. Inspection team members are selected from CLS Health & Safety personnel and senior management. Inspection reports are submitted to the CLS Health and Safety Management Committee for review. Audits performed by an external, independent body are arranged by the CLS Health & Safety Working Group when deemed necessary by the CLS Health & Safety Management Committee. Lab Managers and Biological Safety Advisers are required to continually monitor safety standards and compliance with Health & Safety Policy & Procedures, within their designated area, and report problems and non-compliance to the CLS Health and Safety Working Group.

MSC Testing and Maintenance (TC suites only)

MSCs are serviced and operator protection (KI) tested on an annual basis by a reputable service provider. A certificate of conformity to the required standard is displayed on each cabinet. The Health and Safety Information Officer is responsible for arranging the servicing schedule, ensuring fumigation/decontamination is carried out prior to testing and issuing and keeping a copy of the certificates of conformity. Users are required to perform a visual check on all alarms and indicators before each use and report any defects immediately to their Lab Manager. Note: If an MSC is moved to a new location, or equipment in a room containing a cabinet is significantly re-arranged, to the extent where it may affect the airflows within the room, the cabinet must be KI tested before use to ensure operator protection has not been compromised.

Negative Pressure Testing (TC suites only)

Pressure differentials in TC suites are checked regularly to ensure the suite is at an air pressure negative to the immediate surroundings. Checks are arranged by the CLS Health & Safety Information Officer.

6: Final classification of project

○ Class 1 ◉ Class 2 ○ Class 3

7: Additional information and comments

Vaccinia virus incident described in ACGM Newsletter 32 must be read by all workers. See http://www.hse.gov.uk/biosafety/gmo/acgm/acgm32/paper8.htm