GM Microorganism Risk Assessment

Full Assessment, New Format

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Final Classification of Project: **Class 2**

Title: **Role of Cystatin F in Modulation of CD8 T Cell Response**

Lab: Floor 2 and WBRU CL2 Suite
Bldg: Wellcome Trust Biocentre

1: Brief Description of Project

We are studying the protease inhibitor cysatin F also known as leukocystatin. As its name suggests it is only expressed in immune cells and our work to date indicates that it may attentuate protease activity in CD8 T cells and other immune cells. CD8 T cells can kill other virally infected cells but run the risk of killing themselves preventing normal CD8 T cell 'memory' to the original pathogen. We think that cystatin F may prevent or modulate this so we want to look at CD8 T cell responses to viral antigens in wild type and cystatin F null C57BL/6 mice.

Because the null mice have an H 2b MHC we have to be able to 'read out' a T cell response on that MHC molecule. To achieve this we will use three different strains of influenza A virus with the MHC Kb-restricted immunodominant epitope from HSV-1 glycoprotein B (gB; residues 498 to 505; SSIEFARL) inserted into the neuraminidase stalk: WSN-NAgB, PR8-NAgB and HKx31-NAgB. These recombinant influenza strains were produced by our collaborators in Australia using reverse genetics. This involved inserting SSIEFARL into an expression plasmid containing the viral cDNA of neuraminidase (NA) from influenza A virus strain WSN, PR8 or HKx31. This expression plasmid together with seven expression plasmids containing the other viral cDNAs of influenza A virus (PB1, PB2, PA, NP, M, NS and HA from WSN, HA from PR8 or HA from HKx31, as appropriate) were transfected into eukaryotic cells (MDCK and 293T cells) with the eventual generation of infectious influenza A virus. Recombinant virus stocks were then amplified in 10 day old fertilized chicken eggs.

The mice will be infected and initial CD8 T cell responses measured using special fluorescent probes that quantify the number of peptide specific CD8 T cells that are elicited. The mice will then be rested for several weeks and then rechallenged to measure their memory response.

In vitro work will be carried out in a Containment Level 2 Facility on WTB Floor 2. In vivo work will be carried out in the CL2 facility in the WBRU.

2: Hazards to Human Health

(a) Associated with recipient micro-organism

A generic description of the hazards to human health is given in Microorganism Hazard Assessment 1051. Large differences in pathogenicity between Influenza strains and between variants of any given strain are well documented.

Influenza A virus strains WSN (H1N1) and PR8 (H1N1) were originally isolated from humans in 1933 and 1934 respectively. HKx31 (H3N2) is a laboratory derived, high yielding reassortant of A/PR/8/34 H1N1 (i.e. PR8) and A/Aichi/2/68 H3N2, another human isolate dating back to 1969. Studies in mice have shown that the virulence of X31 is intermediate between that of wild-type human H3N2 isolates and PR8 [1]. All three host strains have been widely used in labs for many years and subjected to prolonged egg and mouse adaptation resulting in decreased infectivity in humans [2]. There are no reported cases of these strains transmitting between mice or between mice and humans.

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

The SSIEFARL epitope is specific for H2Kb which is the MHC I molecule found in C57BL/6 mice. The peptide will not illicit an immune response in humans and there is no evidence to suggest it is inherently harmful to human health.

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

The NA stalk plays a critical role in viral replication, virulence, pathogenesis and species adaption. The impact of stalk length on NA function is not yet clear. However, Castrucci et al have studied the effect of insertion a cytotoxic T-lymphocyte-specific epitope of the LCMV nucleoprotein (residues 116 to 127) into the NA stalk of the WSN (NA15) strain [3]. Two modified strains were created, one in which a portion of the NA stalk was replaced with the LCMV epitope, while in the other, the epitope was inserted into the stalk, lengthening it by 12 amino acids compared with the wild type. Both mutants were comparable with the parent strain in terms of virus production and virulence in mice. Based on this, there is no reason to believe that the SSIEFARL containing mutants will be more virulent than the parent strains.

PR8, WSN and HK31 Neuraminidases are all Inhibited by zanamivir (Relenza) and oseltamivir (Tamiflu). [Info taken from Universal Protein Resource (UniProt) Knowledgebase.] These inhibitors block the active site of the NA molecule, located within it globular head, and prevent it interacting with its natural substrate. There are numerous accounts in the literature of point mutations conferring drug resistance to circulating flu virus strains, but these mutations all occur in and around the catalytic site. Therefore, there is no evidence to suggest that insertion of a short peptide into the NA stalk by itself will confer zanamivir/oseltamivir resistance.

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

The segmented nature of the Influenza genome allows for the exchange of genetic information during mixed infection of cells with different viruses. The progeny that result from such mixed infections, referred to as reassortant viruses, can contain various combinations of gene segments derived from either parental virus. Reassortment between the vast number of circulating strains occurs readily in the natural reservoir.

Transfer of the SSIEFARL peptide from our mutant strains to circulating strains should not have an effect on the hazards status of either virus.

The effect of exchange of other segments of the genome is difficult to predict. Studies using laboratory reassortants showed that neither the HA nor the NA was an exclusive determinant of virulence [4] and that as many as three gene segments may be required for full manifestation of the phenotype [5].

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

Flu virus can survive on environmental surfaces for short periods. If spills are not thoroughly disinfected live virus could be transferred onto clothing, shoes, skin and carried out into the external environment. Also, waste that has not been autoclaved or disinfected correctly could carry live virus into the environment when it is finally disposed of to landfill/drains. Fortunately, flu virus is rapidly inactivated by heat (30 min at 56° C) and various disinfectants including 1% Virkon, 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde, and lipid solvents. Therefore, adhering to our CL2 SOPs should ensure that no live virus is released into the environment. If, however, the GM viruses did escape into, and survive in, the external environment the risk to humans and other organisms, including mice*, would be no greater than that posed by currently circulating wild-type strains. Recombination between a GM virus and wild-type strain could occur if they were to co-infect a host. However, co-infection is just as likely between two circulating strains and the reassortant produced could be less, equally or more pathogenic.

*The parent flu virus strains used in this project have been specifically adapted to infect laboratory strains of mice through serial passage in host animals. Most inbred lab mice are, therefore, highly susceptible to disease following infection; in contrast, wild mice are resistant to infection with these strains. The basis for this difference has been shown to be the lack of expression of a functional Mx1 protein, a critical antiviral factor, in most inbred laboratory mice. [1]

(b) Arising from genetic material

SSIEFARL is specific to the mouse MHC molecule Kb found in the C57BL/6 mice. This is a laboratory strain not found in the external environment. Therefore, the inserted genetic material is unlikely to pose a risk to any host organism.

5: Nature of Work

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

Propagation of the viruses 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 viral stocks 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 intranasally with a small volume of virus.

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

◉ Yes ○ No

(c) Waste Disposal

Standard autoclaving conditions are effective.
1% Virkon is effective (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.

(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?

Vaccination against selected circulating strains of influenza A and B viruses is available but may not be effective against strains used in this research project. Therefore, vaccination is not recommended.

(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

1. N. M. Bouvier and A. C. Lowen. 2010. Animal Models for Influenza Virus Pathogenesis and Transmission. Viruses, 2, 1530-1563

2. A. S. Gambaryan, J. S. Robertson, and M. N. Matrosovich. 1999. Effects of Egg-Adaptation on the Receptor-Binding Properties of Human Influenza A and B Viruses. Virology, 258, 232-239.

3. M.R. Castrucci, S. Hou, P. C. Doherty and Y. Kawaoka. 1994. Protection against Lethal Lymphocytic Choriomeningitis Virus (LCMV) Infection by Immunization of Mice with an Influenza Virus Containing an LCMV Epitope

Recognized by Cytotoxic T Lymphocytes. J. Virol, 68, 3486-3490.

4. V. Mayer, J. L. Schulman and E. D Kilbourne. 1973. Nonlinkage of neurovirulence exclusively to viral hemagglutinin or neuraminidase in genetic recombinants of A/NWS (H0N1) influenza virus. J. Virol, 11, 272-278.

5. A. Sugiura, and M. Ueda. 1980. Neurovirulence of influenza virus in mice. I. Neurovirulence of recombinants between virulent and avirulent virus strains. Virology, 101, 440-449.