

GM Microorganism Risk Assessment

Full Assessment, New Format

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Assessment Reviewed by BSA: Yes

Permission Granted by HoD: Yes

Final Classification of Project: Class 2

Title: Investigating the role of siglecs in the interaction between pathogen and immune response using *Campylobacter Jejuni*

Lab: Floor 2

Bldg: Wellcome Trust Biocentre

1: Brief Description of Project

We are interested in the biology of a family of molecules called siglecs, expressed on cells of the immune system, which bind sialated ligands. Mammalian cells carry many sialated ligands which may signal through siglecs and inhibit any immune response towards itself. Most pathogens are not sialated, however, some human pathogens have gained the ability to mimic host gangliosides, e.g. some strains of *Campylobacter jejuni*, which may provide a mechanism by which the pathogen inhibits the immune system. We wish to study the immune response to both sialated pathogens and non-sialated counterparts to study the role of siglecs in culture and in mice.

This work involves both the propagation of *Campylobacter* in vitro as well as the infection of mice and harvesting of tissues for analysis.

2: Hazards to Human Health

(a) Associated with recipient micro-organism

Campylobacter Jejuni is the most common cause of bacterial gastroenteritis in the western world and causes a self limiting diarrhoeal illness. *C Jejuni* gastroenteritis can be treated with antibiotics although this is only required in a minority of cases. Certain strains of *C Jejuni* are associated with a number of post infectious sequelae such as autoimmune neuropathy (Guillain Barre Syndrome) or reactive arthritis. These affect a small minority of those infected with specific strains of *C Jejuni* (~1%). Some of the wild type organisms used in this project are strains associated with Guillain Barre Syndrome.

C Jejuni is routinely cultured in clinical bacteriology labs on the open bench. The organism is spread by contact, therefore, the main hazard is that the operator's hands are contaminated prior to heat inactivation and that the bacteria are transferred to other surfaces and food. The use of gloves, disinfectant, hand washing and due care and attention is sufficient to reduce this hazard to a minimum. A second hazard is self inoculation by splashes or sharps. The bacteria are only pathogenic if ingested or injected, i.e. skin contact is not an infection risk provided the area is cleaned. Sharps are used only when essential.

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

We have access to various *Campylobacter jejuni* clinical isolates that are either sialated or not; sialated isolates appear to predominate in patients that present with Guillain barre syndrome. We also have access to naturally sialated isolates that have been genetically modified (e.g. CSTII gene knock-out) to lose these sialated ligands. The loss of these genes is not inherently harmful.

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

As a result of the gene deletion, the CSTII KO strains no longer mimic host gangliosides. Theoretically, this should result in there being no "molecular mimicry" between pathogen and host and therefore the GM strains should have less pathogenic potential than wild type strains and theoretically not cause Guillain barre syndrome. The capacity to cause bacterial gastroenteritis may not be affected.

The genetic modifications made to the bacteria are extremely unlikely to affect their transmissibility or susceptibility to disinfectants or heat. It is also unlikely that the modifications would alter sensitivity to antibiotics, i.e. therapeutic interventions are available.

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

As the GM organism is a Knock-out rather than Knock-in strain, the chance of gene transfer does not apply.

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

C Jejuni is a fastidious organism which, when associated with food poisoning, grows on contaminated meat. However, in the laboratory environment C Jejuni has to be grown on selective media in a microaerophilic environment often at higher than normal temperatures in order to prevent overgrowth by competing organisms. C Jejuni will therefore have a low capacity to survive and disseminate from the laboratory (where accidental contamination of food is highly unlikely).

(b) Arising from genetic material

The GM organism is a Knock-out rather than Knock-in strain. There is no reason to believe the GM strain will be any more of a hazard to the environment than the wild type parent strain.

5: Nature of Work

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

Microbiological procedures will be carried out under Containment Level 2 in the general lab and tissue culture suite. For propagation purposes cultures will be grown in volumes of 25-500 mls. Cultures will also be collected for murine infection, or used fixed or unfixed in in vitro experiments. Murine infection will be carried out in a Containment Level 2 facility in the animal unit. Mice will be infected by injection of a small volume of bacteria, and infection monitored in homogenised tissues (typically liver and spleen).

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

Yes No

(c) Waste Disposal

C jejuni is sensitive to moist heat (121°C for at least 15 min).
Virkon is effective against C jejuni 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. Do not use aspirator set-ups.

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 and can spread to animals within the same cage by fecal matter. 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?

None required. However, all those who may be exposed must be familiar with the signs of Campylobacter infection.

(j) Emergency Plan

Small scale cultures and low risk activity.

(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 BSOs 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 rearranged, 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