University of Kentucky (UK)
Institutional Biosafety Committee (IBC)

Date: 02JULY2025
Time: 12:03 PM – 1:46 PM
Location: Virtual Meeting via Zoom - https://uky.zoom.us/j/87284517361

Minutes

Call to Order

The meeting was called to order by Doug Harrison at 12:03PM.

Attendance

IBC Members Present

Steve Brown (Local, Non-Affiliated Member)

Thomas Chambers (Co-Chairperson)

Doug Harrison (Co-Chairperson)

Cheryl Haughton (Animal Containment Expert)

Delphine Malherbe (Laboratory Staff Representative)

Delena Mazzetti (Biological Safety Officer)

Micheal Mendenhall (Institutional Member)

Brandy Nelson (Institutional Member)

Jan Smalle (Plant Containment Expert)

Carol Pickett (Local, Non-Affiliated Member)

Xiangan Li (Institutional Member)

Mindy Thompson (Institutional Member)

Katerina Wolf (Institutional Member)

Regrets

Anika Hartz (Institutional Member)

Arthur Hunt (Plant Containment Expert)

Maj-Linda Selenica (Institutional Member)

Yadi Wu (Institutional Member)

Guests

Elizabeth Brooks (Administrative Support Associate I)

Robert Hayman (Assistant Biological Safety Officer)

Melissa Hollifield (Animal Research Compliance Manager)

Jeff Howell (IBC Administrative Professional II)

Audra Strahl (IBC Administrative Professional II)

Kathryn Childress (Temporary STEPS Office and Clerical)

University of Kentucky (UK)
Institutional Biosafety Committee (IBC)

Quorum

Per the University of Kentucky Institutional Biosafety Committee By-Laws, at least 6 voting members shall constitute a quorum.

Approval of Previous Month's Meeting Minutes

2025.06.04 IBC Meeting Minutes.pdf

Delena Mazzetti requested to include the time at which the June IBC meeting adjourned as a revision to the previous month's meeting minutes.

Thomas Chambers initiated a motion to approve the June 4th, 2025, IBC meeting minutes pending the minor change noted above. Mindy Thompson seconded the motion. All IBC members present (13) voted in favor of the motion.

Old Business

None

New Business

Protocol Review

IBC approval is granted only when biosafety containment and procedures are reviewed and found to be adequate for the research being undertaken and when all biosafety laboratory inspection and training requirements are satisfactorily met. All biosafety laboratory inspection and training requirements are verified by the UK Biological Safety Officer (BSO) or designee prior to final approval. Current UK Biosafety training requirements are available online HERE. Current UK Biosafety Laboratory Inspection Program requirements are available online HERE.

Amendments

PI: Sarah D'Orazio

IBC Protocol Number: IBC-24-42
Protocol Title: Host-pathogen Interactions during Listeria monocytogenes infection
Protocol Type: Amendment
Amendment To: Organisms used in research, Cells or tissues used in research, Personnel
Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-D-4, NIH Guidelines Section III-D-2, NIH Guidelines Section III-F-4, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9
Maximum Containment Level: Biological Safety Level 2 (BSL2)
Primary Reviewers: C. Pickett, K. Wolf, M. Thompson

Brief Project Overview:

Listeria monocytogenes (Lm) infections occur when people eat contaminated processed food products such as unpasteurized cheeses, deli meats and certain produce items. The infections can be deadly, particularly for some pregnant women/neonates and people over the age of 60. In this study, we are testing the idea that Lm elicits a strong protective immune response in most individuals, and that this immune response will limit the infection to a

mild gastroenteritis. We predict that in susceptible individuals who lack the ability to mount this innate immune response, unrestricted growth of the bacteria will result in systemic spread and invasion of the central nervous system.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, Use of Infectious Agents
Transport: Yes
Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Listeria innocua (RG1-bacteria)/Humans, animals; Listeria ivanovii (RG2-bacteria)/Humans, animals; Listeria monocytogenes (RG2-bacteria)/Humans, animals; Listeria spp. (RG2-bacteria)/Humans, animals; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human; Streptococcus mutans (RG2-bacteria)/humans; Staphylococcus aureus (RG2-bacteria)/humans; Burkholderia thailandensis (RG1-bacteria)/soil bacterium
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Cre/bacteriophage/recombinase/heterolgous expression in Listeria/Listeria monocytogenes/pIMC3, pKSV7; gfp/Aequorea victoria/tracking gene/heterologous expression in Listeria/Listeria monocytogenes/pIMC3, pKSV7; various surface attached proteins LPXTG or GW category (see attached sheet)/Listeria monocytigenes/surface attached protein/intermediate subcloning in E. coli prior to complementation of deletion mutants in Listeria/E. coli XL-10 Gold or E. coli SURE (Agilent)/pPL1, pPL2 (only integrates into Listeria genome); mCherry/variant of DsRed derived from Discosoma sea anemone/tracking gene (red)/heterologous expression in Listeria/Listeria monocytogenes/pIMC3, pKSV7
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pPL1; Plasmid/pPL2; Plasmid/pIMC3; Plasmid/pKSV7
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/THP-1; Animal/J774; Human/HepG2; Human/HeLa H1; Animal/bone marrow derived dendritic cells; Animal/N2A; Animal/F11; Animal/SVEC4-10; Animal/EGC; Animal/bEND.3
Animal Use: Yes
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Listeria monocytogenes (RG2-bacteria)/oral feeding, i.g. or i.v./none for feeding; lucite container for i.v./ABSL2/lsecondary ab coat, gloves, shoe covers/ABSL2/Yes/

Risk Assessment/Discussion:

Dr. D'Orazio has submitted an amendment to her existing IBC protocol entitled *Host-pathogen Interactions during Listeria monocytogenes infection*. In this amendment, Dr. D'Orazio has updated administrative information, removed some lab personnel, and is seeking approval to work with *Staphylococcus aureus* (RG2), *Streptococcus mutans* (RG2), and *Burkholderia thailandensis* (RG1). These species will be used as examples of Gram-positive and Gram-negative bacteria in phagocytosis assays in cultured bone marrow derived monocytes, macrophages and dendritic cells. The culture and handling of the strains will be done as described and previously approved for

L. monocytogenes. This work will be completed at BSL2 containment wearing a lab coat, disposable gloves, and eye protection. Dr. D'Orazio has a dedicated lab space with two BSCs for work with these pathogens.

IBC Vote:

The amendment to IBC-24-42 (version 31.0) was approved.
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Kate Wolf initiated the motion. Mindy Thompson seconded the motion. All IBC members present (13) voted in favor of the motion.
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Conflicts of Interest: None
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PI: Emilia Galperin

IBC Protocol Number: IBC-24-154
Protocol Title: Regulation of MAPK activity by EGF receptor endocytosis
Protocol Type: Amendment
Amendment To: Personnel, Manipulations planned
Applicable Guidelines & Regulations: UK Administrative Regulation 6.9, OSHA 29 CFR 1910.1030, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7, NIH Guidelines Section III-F, NIH Guidelines Section III-F-1, NIH Guidelines Section III-D-1, NIH Guidelines Section III-D-4, UK Administrative Regulation 6.3
Maximum Containment Level: Animal Biological Safety Level 1 (ABSL1), Biological Safety Level 2 - Enhanced (BSL2+)
Primary Reviewers: T. Chambers, B. Nelson, D. Malherbe

Brief Project Overview:

The extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling pathway plays an essential role in several critical steps of embryonic development and tumor progression. It also controls critical cellular functions such as apoptosis, motility and differentiation. Kinases and phosphatases of this pathway have been extensively studied and targeted therapeutically. However, the mechanisms that determine the signal specificity and orchestrate the diverse biological outcomes of ERK1/2 signaling are still poorly understood. Scaffold proteins are key players in the ERK1/2 signaling pathway that are thought to integrate incoming signals and deliver signaling specificity, and yet their role in signal propagation and the mechanisms of their action are still unknown. The long-term goal of our research is to reveal how scaffold proteins are involved in the biological processes regulated by the ERK1/2 cascade. In this way, new therapeutic strategies can be developed to more specifically target this cascade without affecting other essential biological functions. In our studies we utilize lentiviruses that express fluorescently tagged proteins and shRNA. Our research protocol involves recombinant DNA techniques, the use of human and animal cell lines, and the use of BSL-2 infectious pathogens, and zebrafish. To minimize risk and maintain a safe working environment, we will follow procedures outlined below.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Use of Viral Vectors, Cell culture, Creation of Viral Vectors, DNA/RNA isolation/purification, PCR/qRT-PCR, Bacterial culture, Transfection, Transformation, Immunohistochemistry, Animal work (breeding, surgeries, etc.), Genetics, Use of Human Source Material(s), Imaging/Microscopy
Transport: Yes
Materials Transported: Animals
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human

Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: EGFR/Human/signal transdusing protein/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10; M-Ras/Human/signal transdusing protein/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10; C-Raf/Human/kinase/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10; Shoc2/Human/signaling modulator/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10, pLVTHM; PSMC5/Human/protein ubiquitilation/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10, pLVTHM; USP7/Human/protein ubiquitilation/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10, pLVTHM; VCP/Human/protein degradation/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10; HUWE1/Human/protein ubiquitilation/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10, pLVTHM; RFP/jellyfish/protein visualization/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10; GFP/jellyfish/protein visualization/expression in cell culture/cell culture (human)/pcDNA3.1, ptaqRFP, pRSETa, pCMV-FLAG10;
Rab5/human/intracellular trafficking protein/expression in cell culture/cell culture (human)/GFP, taqRFP;
Rab7/human/intracellular trafficking protein/expression in cell culture/cell culture (human)/GFP, taqRFP;
Rab11/human/intracellular trafficking protein/expression in cell culture/cell culture (human)/GFP, taqRFP;
Rab4/human/intracellular trafficking protein/expression in cell culture/cell culture (human)/GFP, taqRFP
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/Shoc2-mouse LV/; Plasmid/peXFP (CFP, YFP); Plasmid/pcDNA3.1; Lentivirus/pLTVHM.1; Plasmid/ptaqRFP, ptaqBFP or pTaqGFP; Plasmid/pCMV-FLAG10; Plasmid/psPAX2; Plasmid/pMA2.G; Plasmid/pRSETa; Plasmid/pGEX-4T1
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HeLa; Animal/COS1; Human/293FT; Human/H1703; Human/H1648; Human/T47D; Human/HT29; Human/HT116; Human/MCF7; Human/Jurcat
Animal Use: Yes
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Fish/Naked Nucleic Acid-r/sDNA/Single cell injection/n/a/ABSL1/lab coat, gloves, eye protection/ABSL1/No/N/A

Risk Assessment/Discussion:

Dr. Galperin has submitted an amendment to her existing IBC protocol entitled *Regulation of MAPK activity by EGF receptor endocytosis*. In this amendment, Dr. Galperin has updated administrative information, added lab personnel, clarified previously approved work, and is seeking to prepare lentivirus in her laboratory. Dr. Galperin is currently approved to work with lentivirus targeting Shoc2, HUWE1, PSMC5, and USP7 via shRNA that were originally produced in the UK Genetic Technologies Core laboratory. In this amendment, she is seeking to package/produce the same lentiviruses in her own laboratory. This work will be completed at BSL2+ containment wearing lab coat, disposable gloves, and eye protection. Dr. Galperin has a dedicated cell culture space with a BSC for work with lentivirus. The lentiviruses will be utilized as previously described and approved.

IBC Discussion & Vote:

The amendment to IBC-24-154 (version 29.0) was approved pending minor modifications as listed below:
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RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Viral Vector Information table: Currently marked as "293FT," please update the "Packaging Cell Line," column for Adenovirus to be congruent with the Scientific Summary (listed as HEK293T cells).

CELL LINES – Cells in Use table: Please add the 293 LTV cells mentioned in the Scientific Summary to the table.

DISINFECTANTS, EMERGENCY RESPONSE, TRANSPORT, WASTE – Disinfectants table: The Scientific Summary specifies the use of CIDEX OPA (0.55% o-phthaldehyde) as a disinfectant. Please add this disinfectant to the table.

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Thomas Chambers initiated the motion. Delphine Malherbe seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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New Protocols

PI: Fevzi Yalniz

IBC Protocol Number: IBC-25-57
Protocol Title: Autolus-OOS-EAP: Expanded Access Program (EAP) for Obecabtagene Autoleucel (obe-cel) Out-of-specification (OOS) in Adult Patients with Acute Lymphoblastic Leukemia (AUTO1-OS1)
Protocol Type: New Protocol
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-C-1, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, NIH Guidelines Section III-D-1
Maximum Containment Level: Biological Safety Level 2 (BSL2)
Primary Reviewers: D. Harrison, B. Nelson, T. Chambers

Brief Project Overview:

Obe-cel, Aucatzyl® is used to treat B-cell Acute Lymphoblastic Leukimia (B-cell ALL). Obe-cel, Aucatzyl®, is an FDA-approved treatment when in specification. This EAP (Expanded Access Program) for Obecabtagene Autoleucel (obe-cel) that does not meet the commercial release specifications of Obe-Cel/Aucatzyl® in the United States.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Use of Human Source Material(s), Human Clinical Trial
Transport: Yes
Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: anti-CD19/Human/Chimeric Antigen Receptor/Expression/Autologous Cells/LV18970; CD8 stalk & transmembrane domain/Human/transmembrane domain & structural/Expression/Autologous Cells/LV18970; CD137 cytoplasmic domain (4-1BB)/Human/Cytoplasmic

Domain/Expression/Autologous Cells/LV18970; CD3 zeta cytoplasmic domain/Human/Cytoplasmic Domain/Expression/Autologous Cells/LV18970
Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/LV18970
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: N/A
Animal Use: No
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: N/A

Risk Assessment/Discussion:

Dr. Yalniz has submitted a new IBC protocol for a clinical study entitled *Autolus-OOS-EAP: Expanded Access Program (EAP) for Obecabtagene Autoleucel (obe-cel) Out-of-specification (OOS) in Adult Patients with Acute Lymphoblastic Leukemia (AUTO1-OS1)*. Obe-cell (Aucatzyl) is FDA approved to treat B-cell acute lymphoblastic leukemia (B-cell ALL). This specific study is an Expanded Access Program (EAP) for use of obe-cel that does not meet the commercial release specifications in the US. Obe-cel, is a genetically modified autologous T-cell immunotherapy. In a clinical setting, drug products that do not meet the product specifications for release are sometimes utilized if the Sponsor and Investigator at the clinical site agree that the health of the subject and the risk/benefit profile of the non-conforming product are acceptable. While the risks of using non-conforming Obe-cel are not completely understood, disease prognosis may justify greater risk tolerance. Examples of out-of-specification (OOS) product that may be considered for release through this program include those that do not meet the release specifications for viability, dose (low), CAR expression, viable cell concentration, CAR-T cell function, sterility, and though rare, presence of replication competent lentiviral vector. It is unknown at this time how many patients will be enrolled here at the UK study site, as this will depend on the ability to manufacture Obe-cel from each patient's apheresed cells. Drug product will be received by the UK Cell Therapy Laboratory and stored per manufacturer's specifications prior to infusion. Frozen Obe-cel infusion bags will be inspected before and after transport. Bags will be transported form the UK Cell Therapy laboratory to the Hematology/Bone Marrow Transplant unit at UK Chandler Hospital in labelled, portable liquid nitrogen transport containers. Infusion bags will be thawed at patient bedside in a waterbath and administered to patients by the administering physician within 2 hours of thawing. UK Healthcare personnel will follow all manufacturer's instructions regarding receipt, storage, preparation, administration, and disposal of product. Work with these materials will be completed at BSL2 containment. Personnel handling product will wear lab coat, disposable gloves, eye protection, surgical mask, face shield, surgical gown, booties or shoe covers. The primary risks of working with this study agent are potential exposure to drug product and other human source materials that may contain Hepatitis B, C, D, HIV, or other bloodborne pathogens.

IBC Discussion & Vote:

IBC protocol IBC-25-57 (version 6.0) was returned to the investigator for the significant revisions listed below:
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SCIENTIFIC SUMMARY

1. The release of drug product that is non-sterile and/or contains replication-competent lentivirus could change the risks to a patient, their healthcare providers, and their close contacts. Specifically, will drug

product that is non-sterile and/or contains replication-competent lentivirus be administered to patients? If yes, please address the following questions:

a. How will patients be informed of and consented to the additional specific risks of drug product that is non-sterile and/or contains replication-competent lentivirus?
b. Is there data available for shedding of replication-competent lentivirus, and how are healthcare providers, patients, and family members informed of these risks?
c. How will the risk of shedding replication-competent lentivirus be mitigated, specifically?
d. What additional oversight is in place for the release of non-sterile and/or replication-competent lentivirus containing drug product to patients, beyond the study sponsor and administering physician?
    i. If no other oversight is in place, the UK IBC must be notified of the release of drug product that is non-sterile and/or contains replication-competent lentivirus. This can alter the risk assessment and change how those risks are mitigated at the UK study site.

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Douglas Harrison initiated the motion. Brandy Nelson seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Malinda Spry

IBC Protocol Number: IBC-25-65
Protocol Title: Thomas Hunt Morgan Biology Department Teaching Labs
Protocol Type: New Protocol
Amendment To: N/A
Applicable Guidelines & Regulations: UK Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7, NIH Guidelines Section III-D-4
Maximum Containment Level: Biological Safety Level 1 (BSL1)
Primary Reviewers: D. Harrison, B. Nelson, K. Wolf

Brief Project Overview:

This is for the Department of Biology teaching labs located in the Thomas Hunt Morgan Building. These teaching labs use E. Coli, multiple recombinant yeast strains (Saccharomyces cerevisiae), transgenic fruit flies and organotypic rat hippocampal slice cultures during the semester in the Genetics, Cell Biology and Neuroscience courses.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, Cell culture, DNA/RNA isolation/purification, Genetics, Imaging/Microscopy, PCR/qRT-PCR, Transformation, Animal work (breeding, surgeries, etc.)
Transport: No
Materials Transported: N/A
Infectious Agent(s)/Natural Host(s): N/A
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]:

Beta Galactosidase (lacz)/E.coli/Reporter/Used for blue-white screening method for recombinant E. coli/E.coli/pUC19; Ampicillin resistance (ampR)/plasmid pBR322/LysR transcriptional regulator/Ampicillin Resistance/E.coli/pUC19
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pUC19
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/Organotypic Rat Hippocampus
Animal Use: Yes
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: N/A

Risk Assessment/Discussion:

This is a new IBC protocol registration for the UK Department of Biology Teaching Laboratories, including Genetics Lab BIO 304, Cell Biology Lab BIO 315, and Neuroscience Lab BIO 305. Each course has a distinct set of experiments that students will perform. In the Genetics Lab BIO 304, students will utilize *Saccharomyces cerevisiae* and attempt to identify different yeast mutations. Students will wear gloves, goggles, and lab coats while participating in these experiments. Lab surfaces will be wiped with 70% ethanol at the end of each lab period. Yeast, plates, broth, and other disposables will be disposed of as biohazardous waste for autoclaving and disposal by the lab coordinator. The Genetics lab will also utilize pUC19 plasmid vector for heat-shock transformation into NEB 5-alpha *Escherichia coli*. PUC19 expresses beta-galactosidase (lacz) and ampicillin resistance (ampr). Students will wear gloves, lab coats, and eye protection. Lab bench surfaces will be wiped with 70% ethanol at the end of each lab session. All plates and other lab materials will be disposed of as biohazardous waste for autoclaving and disposal by the lab coordinator. Students will also learn to perform restriction digests and ligation reactions, and select white and blue *Escherichia coli* colonies for isolation of plasmid DNA. The Genetics and Cell Biology courses also utilize transgenic fruit flies propagated by the Biology Department. Flies are anesthetized with CO2, counted, and sexed for breeding. Embryos will be observed for expression of reporter gene using fluorescence microscopy. All fruit flies are killed via freezing prior to disposal. Fly traps are setup throughout to catch and kill any escaped flies. The Neuroscience lab utilizes rat hippocampus brain slices and cells from Dr. Mark Prendergast's laboratory. These are harvested from neonatal rats that contain no transgenic materials, pathogens, or other biohazardous materials. Although this specific project does not involve the use of biohazardous materials, student will wear gloves, lab coats, and eye protection during lab work. Lab surfaces will be decontaminated with 10% bleach for 20 minutes after each lab session is completed. Overall, the biohazardous risks associated with these teaching lab classes is minimal. BSL1 containment is utilized throughout.

IBC Discussion & Vote:

The protocol IBC-25-65 (version 12.0) was approved pending minor modifications as listed below:
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SCIENTIFIC SUMMARY

1. The use of 10% bleach followed by 70% EtOH should be utilized for the disinfection of surfaces and equipment related to all work associated with *E. Coli*. Please note this in manipulations and the scientific summary.

2. Please comment on what expected environmental impacts, if any, could result from the release of these transgenic organisms.
3. Remove the description of neurobiology lab procedures associated with rat brains as these are not considered biohazardous materials and are not subject to IBC registration.
4. Please clearly state that TAs will be required to take institutional Biological Safety Training offered on SciSure in addition to PIs and other staff working in the labs.

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Kate Wolf initiated the motion. Brandy Nelson seconded the motion. Doug Harrison abstained from voting. All IBC members present (12) voted in favor of the motion.

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Conflicts of Interest: Doug Harrison, who is listed as a co-investigator on this protocol.

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PI: Malinda Spry

IBC Protocol Number: IBC-25-69
Protocol Title: Biology Teaching Labs-Microbiology BIO209 and BIO309
Protocol Type: New Protocol
Amendment To: N/A
Applicable Guidelines & Regulations: UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7
Maximum Containment Level: Biological Safety Level 2 (BSL2)
Primary Reviewers: D. Harrison, B. Nelson, K. Wolf

Brief Project Overview:

Throughout the semester these labs use various strains of bacteria for learning purposes. They grow, test and stain these bacteria.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, Imaging/Microscopy, Histology, Propagation of infectious agents, Use of infectious agents
Transport: No
Materials Transported: N/A
Infectious Agent(s)/Natural Host(s): Staphylococcus epidermidis (RG1-bacteria)/Human Skin Microbiota; Serratia marcescens (RG2-bacteria)/Environmental, soil, water, plants; Micrococcus luteus (RG1-bacteria)/Soil, dust, water, air; Escherichia coli (RG2-bacteria)/GI tract of many animals including humans; Enterobacter aerogenes (RG2-bacteria)/Human and Animal GI tract; Proteus vulgaris (RG2-bacteria)/GI tract humans and animals; Pseudomonas aeruginosa (RG2-bacteria)/Environmental: soil, water, plants; Staphylococcus aureus (RG2-bacteria)/human, livestock, companion animals, wild animals; Bacillus cereus (RG2-bacteria)/Soil, vegetation, Intestinal tracts of some insects and animals; Enterococcus faecalis (RG2-bacteria)/Human GI tract; Streptococcus agalactiae (RG2-bacteria)/GI and Vaginal tracts of humans and bovine species; Streptococcus pyogenes (RG2-bacteria)/Human skin and throat; Streptococcus pneumoniae (RG2-bacteria)/Upper respiratory tract of humans; Klebsiella pneumoniae (RG2-bacteria)/Human and mouse GI tract and upper respiratory tract; Bacillus subtilis (RG1-bacteria)/Soil, dust, GI tract of animals; Unknown Diagnostic Samples/Unknown Water and

Soil Diagnostic Samples
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: N/A
Vector(s) [Vector Category/Vector Technical Name]: N/A
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: N/A
Animal Use: No
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: N/A

Risk Assessment/Discussion:

This is a new IBC protocol registration for the UK Department of Biology Microbiology Teaching Laboratories, BIO 209 and BIO 309. These courses will utilize a number of RG1 and RG2 bacterial organisms in the course of their studies, including *Staphylococcus epidermidis* (RG1), *Serratia marcescens* (RG2), *Micrococcus luteus* (RG1), *Escherichia coli* (RG2), *Enterobacter aerogenes* (RG2), *Proteus vulgaris* (RG2), *Pseudomonas aeruginosa* (RG2), *Staphylococcus aureus* (RG2), *Bacillus cereus* (RG2), *Enterococcus faecalis* (RG2), *Streptococcus agalactiae* (RG2), *Streptococcus pyogenes* (RG2), *Streptococcus pneumoniae* (RG2), *Klebsiella pneumoniae* (RG2), and *Bacillus subtilis* (RG1). These organisms are purchased from Carolina Biologicals and prepared and maintained by Teaching Lab Assistants throughout each semester. Students will be trained by graduate TAs and undergo various biosafety assignments prior to beginning work in the lab. Students will practice aseptic technique and learn to propagate bacteria for bacterial smears on slides for microscopy. Students will wear gloves, lab coats, and eye protection. Students will utilize different media to differentiate temperature and oxygen requirements for bacterial growth. They will also obtain water and soil samples from the surrounding environment and attempt to identify bacteria. Benchtops will be wiped with freshly prepared 10% bleach at the start and end of every lab session. All biohazardous waste is collected by the lab coordinator for autoclaving and disposal. All work, regardless of the bacterial agents in use, will be done at BSL2 containment. The work of these courses does not involve any recombinant or synthetic nucleic acid materials or animals.

IBC Discussion & Vote:

The protocol IBC-25-69 (version 18.0) was approved pending minor modifications as listed below:
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INFECTIOUS AGENTS: Please clarify how the specific total culture volume of 640mL was ascertained.
SCIENTIFIC SUMMARY:

1. Please specify that lab notebooks and writing implements must remain in the lab and are not taken home with students.
2. In the attached training file, please note that disposable gloves are worn consistently during manipulations with biohazardous materials. This is consistent with CDC BMBL 6th edition practices and procedures.
3. In the attached training file - many industrial, institutional, and clinical labs no longer allow the presence of open flames. Please update procedures noting open flame usage to also reflect non-open flame methods, that more accurately reflect future lab environments in which students may be employed.

4. Are students instructed to report potential exposures and/or injuries to the instructors/TAs/GAs/lab supervisor? What protocol is provided to students seeking care in the event of exposure/illness? Please reference the flow chart here and include it in the student's initial orientation.
5. Please also ensure students are informed that individual health status may impact their susceptibility to disease should they be exposed to the biohazardous agents in use.
6. Who is responsible for cleaning student lab coats? How is this accomplished?
7. While it is an important part of learning to work safely in the laboratory, please also add that final cleaning and disinfection at the end of each class period will also be completed by a TA or lab coordinator.
8. Are there safer attenuated alternatives for the bacterial pathogens in use? A discussion of this and justification for use of the bacterial pathogens listed should be included here.

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Doug Harrison initiated the motion. Kate Wolf seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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Renewals

PI: Preetha Shridas

IBC Protocol Number: IBC-25-59
Protocol Title: Inflammation, lipoproteins and chronic disease.
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-D-1, NIH Guidelines Section III-D-4, NIH Guidelines Section III-E-1, NIH Guidelines Section III-E-3, NIH Guidelines Section III-F, NIH Guidelines Section III-F-1, NIH Guidelines Section III-F-2, NIH Guidelines Section III-F-4, UK Administrative Regulation 6.9, UK Administrative Regulation 6.3, OSHA 29 CFR 1910.1030, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section IV-B-7
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)
Primary Reviewers: C. Haughton, X. Li, D. Malherbe

Brief Project Overview:

Inflammation plays a major role in many chronic diseases, including cardiovascular disease, atherosclerosis, insulin resistance, and diabetes. Our laboratory studies several aspects of inflammation, including how inflammation alters the metabolism of high-density lipoprotein (HDL, the "good cholesterol" in blood) and low-density lipoprotein (LDL, the "bad cholesterol" in blood). A major goal is to understand how various proteins, whose concentration in the blood is increased during inflammation, alter lipoprotein structure and function. Of particular interest is serum amyloid A (SAA), a protein found associated with HDL in the blood during inflammation. Our study focuses on investigating the roles of SAA in both chronic inflammation, such as atherosclerosis, and acute inflammation, such as sepsis. We also study how inflammation alters the interaction of lipoproteins with liver cells and immune cells.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Flow cytometry/Cell sorting, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Transfection, Use of Human Source Material(s), Use of viral vectors, Creation of viral vectors, Genetics, Propagation of infectious agents, Transformation, Use of infectious agents, Viral culture
Transport: Yes
Materials Transported: Animals, Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Luciferase/Firefly/reporter/Expression in cell culture, mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) /any mammalian cell line/pTK-luc; Liver X Receptor/mouse//Expression in cell culture, mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) /any mammalian cell line/pGL4.74; Retinoid X receptor/mouse//Expression in cell culture/any mammalian cell line/pGL4.74; Group V secretory phospholipase A2/mouse, human/codes for lipolytic enzyme/Expression in cell culture/any mammalian cell line/pcDNA3.0; Group X secretory phospholipase A2/mouse, human/codes for lipolytic enzyme/Expression in cell culture/any mammalian cell line/pcDNA3.0; Myc-DDK-tagged ORF clone/mouse/enzymatic/Expression in cell culture/any mammalian cell line/pGL4.74; Mus musculus furin /mouse/enzymatic/Expression in cell culture, mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagent/any mammalian cell line/pcDNA3.0; mouse Serum Amyloid A1.1/mouse/structural protein/expression of SAA1 in eukaryotic cell lines; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) /any mammalian cell line/pcDNA3.0; mouse Serum Amyloid A2.1/mouse/structural protein/expression of SAA2 in eukaryotic cell lines; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) /any mammalian cell line/pcDNA3.0; mouse Serum Amyloid A1.1,2.1,CEJ and mutants/mouse/structural protein/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents, and by adenoviral , adeno-associated viral or antisense oligos in cells and mice./mice/adeasy; Serum Amyloid A 1 and 2/human/structural protein/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents. and by adenoviral , adenoassociated vir/mammalian cells/pcDNA3.0; cholesterol ester transfer protein/human/transfer protein/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents and by adenovirus and adeno associated virus in mice./mice, mammalian cells/adenoviral vector; adeno associated virus; Phospholipid transfer protein/human/transfer protein/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents and by adenovirus in mice./mice, mammalian cells/adenoviral vector; Endothelial lipase/human/lipolytic enzyme/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents and by adenovirus in /mice, mammalian cells/adenoviral vector; hepatic lipase/human/lipolytic enzyme/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents and by adenovirus

in /mice, mammalian cells/adenoviral vector; Scavenger Receptor Class B Type 1/human/membrane protein/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents and by adenovirus in mice./mice, mammalian cells/adenoviral vector; Toll like Receptor 4/mouse/membrane protein/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents and by adenovirus in /mammalian cells/pcDNA3.0; MyD88/mouse/adaptor protein/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents and by adenovirus in /mammalian cells/pcDNA3.0; human myeloperoxidase/human/enzyme involved in oxidation reactions/expression in bone marrow derived cells of mice. /mammalian cells/MSCV; Group II A secretory phospholipase A2/mouse/lipolytic enzyme/expression in animals by adeno associated viruses/mice/adeno associated viruses; Serum Amyloid A1/human/luciferase reporter/express luciferase protein in response to human SAA1 promoter activities/mammalian cells/pTK-luc; Serum Amyloid A3/mouse/luciferase reporter/express luciferase protein in response to mouse SAA3 promoter activities/mammalian cells/pTK-luc; PPAR alpha/mouse/luciferase reporter/express luciferase protein in response to mouse PPAR alpha promoter activities/mammalian cells/pTK-luc; Comparative gene identification 58 (CGI-58)/mouse/lipid-droplet associated protein involved in triglyceride hydrolysis/antisense oligonucleotide administration to suppress CGI-58 expression/mice/anti-sense oligonucleotide (ASO); Adipose tissue triglyceride lipase (ATGL)/mouse/Enzyme involved in triglyceride lipolysis/antisense oligonucleotide administration to suppress ATGL expression/mice/anti-sense oligonucleotide (ASO);
Apolipoprotein-B (ApoB)/mouse/Protein essential for the assembly and secretion of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL)/antisense oligonucleotide administration to suppress ApoB expression/mice/anti-sense oligonucleotide (ASO); Serum Amyloid A 1/mouse/Structural and acute-phase protein/antisense oligonucleotide administration to suppress SAA expression/mice/anti-sense oligonucleotide (ASO); Serum Amyloid A (SAA)*/mouse/Structural and acute-phase protein/expression of human SAA /mice/adeno viral vector; Serum Amyloid A mutant1*/mouse/Structural and acute-phase protein (residues 29-42 mutated/expressing mutant hSAA/mice/adeno viral vector; Serum Amyloid A mutant2*/mouse/Structural and acute-phase protein (residues 39-41 mutated/expressing mutant hSAA/mice/adeno viral vector; Serum Amyloid A mutant2*/mouse/Structural and acute-phase protein (residues 77-104 deleted/expressing mutant hSAA/mice/adeno viral vector;
PCSK9/mouse/Increases plasma cholesterol levels by blocking LDL receptor/expression in cell culture and animals; mode of introduction by transfection of plasmids using liposome based (example, Lipofectamine) transfection reagents and by adenovirus and adeno associated virus in mice/mammalian cells or mice/Adeno-associate virus
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/pTA-luc; Plasmid/pCMX; Plasmid/pCMV; Plasmid/pGL4.74; Plasmid/pTK-luc; Plasmid/pFN26A(Bind); Plasmid/pGL4.35; Plasmid/pBIND-GR; Plasmid/pGL2; Plasmid/pShuttle-CMV; Plasmid/AdEasy; Plasmid/pcDNA 3.0; Plasmid/pcDNA3.1; Plasmid/pCMV5.0; Plasmid/pCMV5; Plasmid/pCMV6-Kan/Neo; Plasmid/PS100001; Naked nucleic acid/antisense oligonuleotide SAA1.1; Naked nucleic acid/antisense oligonuleotide SAA2.1; Naked nucleic acid/antisense oligonucleotide scrambled; Plasmid/pGIPZ lentiviral plasmid; Plasmid/pEF6/V5-His-TOPO; Adeno-Associated Virus (AAV)/AAV2/8 chimeric vector; Adeno-Associated Virus (AAV)/AAV8 chimeric vector; Naked nucleic

acid/antisense oligonucleotide for apolipoprotein B (apoB); Naked nucleic acid/antisense oligonucleotide for CGI-58 protein; Naked nucleic acid/antisense oligonucleotide for ATGL protein; Adenovirus/pAdVector
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/J774; Animal/RAW; Animal/Cos-7; Animal/CHO-1C6; Human/HEK-293; Animal/3T3-L1; Animal/Y-1; Animal/OP9; Animal/p388D1; Human/NCI-H295R; Human/Hep G2; Animal/Min-6; Animal/Lewis lung carcinoma cell line; Animal/CT26 mouse colon cancer cell line; Human/289; Human/SW-872 (HTB-92); Human/JKW; Animal/BHK; Human/THP1; Animal/FU5AH; Human/HEK-Blue; Animal/Murine bone marrow cells; Animal/AML12
Animal Use: Yes
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Viral Vector – Adenovirus/Tail-vein injection/Plexiglass restrainer/ABSL2/Eye protection, disposable gloves, sleeve-cover, lab coat, head and foot covers, gown/ABSL2/No/The animal experiments are performed in BSL2+ facility at the DLAR animal facility; Mouse/Viral Vector - Adeno-Associated Virus (AAV)/intraperitoneal/no restrain/ABSL1/disposable gloves, lab coat, gown, eye protection/ABSL1/No/No/special housing required; Mouse/Naked Nucleic Acid-r/sDNA/IP injection/no restrain/ABSL1/Lab coat, gloves, gown, eye protection/ABSL1/No/No/special housing required

Risk Assessment/Discussion:

Dr. Shridas has submitted a renewal of her existing IBC protocol entitled Inflammation, lipoproteins and chronic disease. Her laboratory studies aspects of inflammation and how inflammation alters the metabolism of high-density and low-density lipoprotein. A major goal of Dr. Shridas' laboratory is to understand how various proteins alter lipoprotein structure and function during inflammation. They are particularly interested in serum amyloid a (SAA), a protein that has been found to be associated with HDL in the blood during inflammation. This requires several experimental approaches including mammalian cell culture, various recombinant/synthetic nucleic acid materials including plasmid transfection, antisense oligonucleotides (ASOs), and viral vectors (adenovirus and adeno-associated virus), in addition to animal models. They will also obtain de-identified human plasma from commercial sources for analysis of HDL, SAA, and CETP levels via ELISA analysis. Adenovirus expressing human SAA mutants lacking key neutrophil-binding domains will be obtained from Vector Biolabs packaged and ready for use, however Dr. Shridas does wish to maintain the ability to package and concentrate adenovirus vectors in the future, if necessary. This work is performed under BSL2+ containment, including all work being performed in the BSC (no open bench work), use of lab coats, liquid-resistant disposable gowns with knit cuffs, gloves, and eye protection. Adenovirus will be transported from their primary laboratory in sealed secondary containers to the animal facility for tail-vein administration within a BSC. Mice are placed into an opaque restraint chamber for tail-vein injections, which greatly enhances the safety for animal handlers administering adenovirus. Mice will be housed at ABSL2 containment. Mice will also be administered AAV expressing SAA1, SAA2, SAA3, CETP, group IIA sPLA2 or PCSK9 via intraperitoneal injection. Similarly, antisense oligonucleotides (ASOs) targeting CGI-58, ATGL, apolipoprotein B, SAA, or control will be administered to mice via intraperitoneal injection. Animal work with AAV and ASOs is completed using ABSL1 experimental procedures and housing. RNA and proteins will be isolated from animal tissues and human plasma. Lab personnel will wear lab coats, gloves, face shields, and safety glasses when homogenizing tissue samples. Lipoprotein particles (Chylomicrons, VLDL, LDL, HDL) will be isolated from mouse plasma via ultracentrifugation using sealed tubes. While not active, Dr. Shridas' laboratory does have a

project utilizing 125[I], 14[C], and 3[H] for labeling proteins. This work takes place in a dedicated BSC reserved for use with radioactive materials.

IBC Discussion & Vote:

The protocol IBC-25-59 (version 8.0) was approved pending minor modifications as listed below:
*
ANIMAL RESEARCH – Animals with Biohazards table: Please update the table to state “manual restraint” instead of “no restraint” for injection of biohazardous agents into mice.
RECOMBINANT and/or SYNTHETIC NUCLEIC ACID MATERIALS – Gene Information table: Please update the "Gene Category” for the "Liver X Receptor” and "Retinoid X receptor” entries.
SCIENTIFIC SUMMARY:

1. Please describe whether harvested and processed tissues are from mice exposed to viral vectors are being handled inside a biosafety cabinet or on the benchtop.
2. Regarding ELISA processing on the open bench, please explain whether human plasma samples are heat inactivated before handling.
3. Please expand on the description of how radioactive waste will be disposed beyond “in accordance with standards set up by UK Radiation Safety Office”.

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Delphine Malherbe initiated the motion. Xiangan Li seconded the motion. All IBC members present (13) voted in favor of the motion.
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Conflicts of Interest: None
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PI: Young-Sam Lee

IBC Protocol Number: IBC-25-64
Protocol Title: Cellular metabolites regulating cancer cell adaptation
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, NIH Guidelines Section IV-B-7, NIH Guidelines Section III-D-1
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)
Primary Reviewers: C. Haughton, C. Pickett, M. Thompson

Brief Project Overview:

The goal of my research program is to determine the contribution of altered metabolism to human diseases. We use biochemical, cellular, and animal-based experiments to achieve this goal. In particular, we are interested in the role of de novo purine nucleotide biosynthesis, pentose phosphate pathway, and nuclear hormone receptors on cancer. Genes involved in de novo purine nucleotide synthesis, pentose phosphate pathway, and nuclear hormone receptors will be either overexpressed or knockdown, followed by cellular and xenograft experiments.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Bacterial culture, Cell culture, DNA/RNA isolation/purification, PCR/qRT-PCR, Use of Human Source Material(s), Use of viral vectors, Flow cytometry/Cell sorting
Transport: Yes

Materials Transported: Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: PGD/Human/Metabolic enzyme/Expression/E. coli/PET series vectors; adenylosuccinate lyase (ADSL) /Human/metabolic enzyme/expression/silencing /Human Cells/lentiviral vector; glucose-6-phosphate dehydrogenase (G6PD) /Human/metabolic enzyme/expression/silencing /Human Cells/lentiviral vector; 6-phosphogluconate dehydrogenase (6PGD) /Human/metabolic enzyme/expression/silencing /Human Cells/lentiviral vector; Nuclear receptor subfamily 2 group E member 3 (NR2E3) /Human/regulatory protein/expression/silencing /Human Cells/lentiviral vector; PAICS/Human/Metabolic enzyme/expression/E. coli/pET series vectors;
PGD/Human/Metabolic enzyme/Expression/E. coli/PET series vectors; G6PD/Human/Metabolic enzyme/Expression/E. coli/pET series vectors; green fluorescent protein (GFP)/Aequorea victoria /GFP Tracking Gene/expression/Human Cells/lentiviral vector
Vector(s) [Vector Category/Vector Technical Name]: Lentivirus/piLenti-GFP (from ABM) and derivatives
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/HeLa; Human/HepG2; Human/H1299; Human/Y79; Human/HEK293; Human/NIH 3T3; Human/A549
Animal Use: Yes
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Cells - Human, genetically modified/SubQ/Isoflurane anesthesia/ABSL2/Lab coat, gloves, eye protection, head cover/ABSL1/No/N/A

Risk Assessment/Discussion:

Dr. Lee has submitted a renewal of his existing IBC protocol entitled Cellular metabolites regulating cancer cell adaptation. His current IBC protocol will expire on July 16, 2025. Dr. Lee's laboratory seeks to understand the role of succinyl-5-aminoimidazole-4-carboxamide-1-ribose-5'-phosphate (SAICAR), a metabolite in the de novo purine nucleotide biosynthesis pathway, in cancer cells. Dr. Lee's laboratory utilizes 3 different types of experiments towards this aim – recombinant protein expression and purification, genetic manipulation of mammalian cells via lentivirus, and xenograft models in mice. In the first category of experiments, E. coli BL21 will be utilized to express recombinant human metabolic enzymes (PAICS, PGD, and G6PD) via pET series plasmid vectors. Proteins will be purified by affinity chromatography. In the cellular experiments planned, Dr. Lee's laboratory will utilize lentiviral vectors obtained from commercial sources (ABM or Genecopoeia) to overexpress or knock down human metabolic enzymes or regulatory proteins (PAICS, ADSL, G6PD, 6PGD, NR2E3) in mammalian cells. GFP will also be expressed via lentivirus for tracking gene expression. The lentivirus in use is a 3rd generation lentivirus that utilizes the VSV-g envelope glycoprotein, making this an amphotrophic lentivirus. Lentivirus is packaged by vendors, significantly reducing risk to Dr. Lee's laboratory. Transduced cells will be grown and harvested to characterize the effects of the genetic modifications to various cellular properties via Western analysis, microscopy, and mass spectrometry. Prior to these manipulations, cells are lysed using SDS buffer, fixed with 4% paraformaldehyde, or metabolites extracted using 99% methanol. Dr. Lee specifies that all work with lentivirus and transduced cells will be completed within a Biological Safety Cabinet (BSC) or sealed containers. The

lentiviral transgenes are not known oncogenes, however overexpression of some have been observed in some tumor types. Transduced cells will be transported to the Flow Cytometry and Cell Sorting Facility in accordance with their IBC protocol, IBC-24-495. Lentivirus transduced cells will be selected for 2 weeks prior to animal experiments. Cells will be transported to the animal facility in sealed secondary containers for subcutaneous injection into anesthetized mice. Mice will be euthanized at the conclusion of study and tumor tissues obtained for downstream assays as described above for cellular experiments (ex. Western blot, mass spectrometry). All manipulations of potentially infectious materials are described as being completed within a BSC, and lab members are required to wear lab coats, gowns, gloves, and eye protection. All work with lentivirus will be completed in a dedicated tissue culture room using BSL2+ containment. Administration of modified human cells will take place under ABSL2 experimental conditions, and animals will be housed at ABSL1 housing.

IBC Discussion & Vote:

The protocol IBC-25-64 (version 10.0) was approved.

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Carol Pickett initiated the motion. Mindy Thompson seconded the motion. All IBC members present (13) voted in favor of the motion.

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Conflicts of Interest: None

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PI: Octavio Gonzalez Duque

IBC Protocol Number: IBC-25-70
Protocol Title: Effect of oral bacteria on innate epithelial responses
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section III-F-1, NIH Guidelines Section IV-B-7, OSHA 29 CFR 1910.1030, OSHA Act of 1970 Clause 5(a)(1), UK Administrative Regulation 6.3, UK Administrative Regulation 6.9
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 2 (ABSL2)
Primary Reviewers: C. Haughton, M. Mendenhall, C. Pickett

Brief Project Overview:

(1) Antimicrobial and inflammatory epithelial responses modulated by P. gingivalis.
Oral infections associated with the gingival margin and subgingival sulcus leads to host inflammatory responses. Gingivitis affects nearly everyone in the population worldwide, at some time during their life. Periodontitis is a disease that encompasses the hard and soft tissue, microbial colonization, and inflammatory responses. While these diseases are not considered to result in direct mortality or substantial morbidity, they have economic and societal detriments on affected individuals. These studies aim to identify early events associated with the pathogenesis of periodontal disease involving bacteria-oral epithelial cells interactions leading to dysbiosis and inflammation using cell cultures and a validated P. gingivalis oral gavage mice model. This work can lead to identify future targets to prevent dysbiosis in the oral and other mucosal surfaces leading to chronic inflammatory disease. (2) Modulation of innate epithelial cell responses by oral commensal bacteria.
The mechanisms through which a persistent recognition of oral commensal bacteria by oral epithelial cells (OECs) mitigates an uncontrolled inflammatory response of the oral mucosa remain unknown. Evidence suggests that

epithelial cells may discriminate between commensals and pathogenic bacteria through differential activation of responses, particularly cytokine/chemokine patterns, which ultimately influence both innate and adaptive arms of the immune response. These studies seek to identify mechanisms of active regulation of inflammatory responses in OECs by specific miRNAs as a potential "tolerogenic mechanism" by which oral commensal bacteria maintain symbiosis with the host. This work involves mainly cell cultures of immortalized oral epithelial cells co-cultured with oral commensal and pathogenic bacteria to determine chemokine transcriptional and translational responses as well as variations in the expression of miRNAs by oral epithelial cells in response to the different challenges.
(3) Study HIV promoter reactivation by oral bacteria in HIV-latently infected cells.
HAART (Highly Active Antiretroviral Therapy), a triple drug therapy (nucleoside reverse transcriptase inhibitor: NRTI; protease inhibitor: PI; non-nucleoside reverse transcriptase inhibitor: NNRTI) is the standard of care to manage HIV infections. In spite of the initial positive response to HAART, patients frequently lose responsiveness to the prescribed regimen. The persistence of HIV-1 latently infecting resting CD4+ T cells, macrophages and dendritic cells constitutes a major obstacle in the control of HIV-1 infection. Importantly various exogenous stimuli have been shown to reactivate HIV-1 infections. Microbial co-infections have been associated with transient bursts of HIV-1 viremia in patients. This can result from microbial activation of pro-inflammatory cytokines, with subsequent induction of HIV-1 production by infected T cells, macrophage (BF-24), and/or dendritic cells (THP-1 derived). Periodontitis is a polymicrobial disease that represents a chronic immunoinflammatory lesion that undermines soft tissue integrity and progresses to resorption of alveolar bone. The lesion is a result of a complex host response comprising inflammatory cells, cytokines, chemokines, and mediators produced by resident gingival cells and inflammatory cells that chemotax into the infected tissues. It is clear that a polymicrobial challenge derived from subgingival biofilms triggers this response in local host tissues. While it is postulated that there is a potential biologic link between periodontal diseases and HIV reactivation, minimal data is available elucidating how oral pathogens could play an important role in HIV reactivation. Currently, there is no cell model available to study the interaction between oral bacteria and HIV latently-infected DCs. We have developed a cell model of HIV-latently infected DCs and used this model to determine if microorganisms from oral biofilms can activate HIV-1 in latently infected cells.

Summary of Biohazard Materials & Manipulations:
Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Histology, Immunohistochemistry, Imaging/Microscopy, Transfection, Use of infectious agents, PCR/qRT-PCR, Use of Human Source Material(s), Flow cytometry/Cell sorting
Transport: Yes
Materials Transported: Animals
Infectious Agent(s)/Natural Host(s): Actinomyces naeslundii (RG2-bacteria)/human; Aggregatibacter actinomycetemcomitans (RG2-bacteria)/human; Campylobacter rectus (RG1-bacteria)/human; Capnocytophaga sputigena (RG2-bacteria)/human; Fusobacterium nucleatum (RG2-bacteria)/human; Listeria monocytogenes (RG2-bacteria)/human; Neisseria mucosa (RG2-bacteria)/human; Prevotella intermedia (RG2-bacteria)/human; Porphyromonas gingivalis (RG2-bacteria)/human; Streptococcus gordonii (RG2-bacteria)/human; Streptococcus mutans (RG2-bacteria)/human; Streptococcus sanguinis (RG2-bacteria)/human; Tannerella forsythia (RG2-

bacteria)/human; Veillonella parvula (RG1-bacteria)/human; Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/human; Lacticaseibacillus rhamnosus (RG1-bacteria)/human; Acinetobacter baumanii (RG2-bacteria)/human; Candida albicans (RG2-fungus)/human
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Notch-1/Human/Cell growth & division/knockdown via siRNA/Cells/N/A
Vector(s) [Vector Category/Vector Technical Name]: Naked nucleic acid/siRNA knockdown of Notch-1
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/THP89GFP; Human/THP-1; Human/BF-24; Human/TIGK cells; Human/OKF4/OKF5 cells; Animal/IMOK mice cells
Animal Use: Yes
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Porphyromonas gingivalis (RG2-bacteria)/Gavage/Hand held/ABSL2/Lab coat, gloves, eye protection, face mask, disposable gowns and booties/ABSL2/Yes/To be performed in BSC in DLAR housing/use room.

Risk Assessment/Discussion:

Dr. Gonzalez Duque has submitted a renewal of his IBC protocol entitled *Effect of oral bacteria on innate epithelial responses*. His existing IBC protocol will expire on August 18, 2025. Dr. Gonzalez' laboratory seeks to identify early events associated with pathogenesis of periodontal disease involving bacteria-oral epithelial cells interactions leading to inflammation and disease by utilizing a P. gingivalis oral gavage mouse model. They will also pursue cell culture studies that involve the co-culture of oral commensal and pathogenic bacteria to determine transcriptional and translational responses and variation in miRNA expression. In a third project, Dr. Gonzalez studies the interaction between oral bacteria and HIV latently-infected cells to determine if oral biofilms can activate HIV-1 in latently infected cells. Dr. Gonzalez' laboratory utilizes a number of RG2 and some RG1 bacterial pathogens that are largely associated with the human oral flora and periodontal disease. These pathogens include, *Actinomyces naeslundii* (RG2), *Aggregatibacter actinomycetemcomitans* (RG2), *Campylobacter rectus* (RG1), *Capnocytophaga sputigena* (RG2), *Fusobacterium nucleatum* (RG2), *Listeria monocytogenes* (RG2), *Neisseria mucosa* (RG2), *Prevotella intermedia* (RG2), *Porphyromonas gingivalis* (RG2), *Streptococcus gordonii* (RG2), *Streptococcus mutans* (RG2), *Streptococcus sanguinis* (RG2), *Tannerella forsythia* (RG2), *Veillonella parvula* (RG1), *Lacticaseibacillus rhamnosus* (RG1), *Candida albicans* (RG2), and *Acinetobacter baumanii* (RG2). All work with the bacterial agents listed will be conducted at BSL2 containment. Lab personnel will wear a lab coat, eye protection, and gloves. Although many of the bacterial agents are considered normal inhabitants of the human oral flora, work with these materials will be completed within a BSC. An anaerobic chamber is utilized for the growth of anaerobic bacteria. For their in vivo studies, periodontitis will be induced by administration of bacterial agents to mice via oral gavage of *Porphyromonas gingivalis*. The oral gavage feed needle has a blunt end, significantly reducing chance for accidental needlestick. They will also utilize a modified ligature-induced model of periodontitis in mice to induce inflammation and bone loss. Oral gavage administration of *Porphyromonas gingivalis* will be done in a BSC in DLAR in a dedicated ABSL2 procedure room. Mice will be housed at ABSL2 for the duration of this study. Bacteria are transported from the laboratory to DLAR spaces in a leak-proof, shatter-proof container. Mice will be transported back to the primary laboratory for euthanasia and tissue collection. They will also utilize gingiva tissues obtained from transgenic mice overexpressing sPLA2-IIA that will be co-culture with

Listeria monocytogenes and *Acinetobacter baumanii* to evaluate the ability of sPLA2-IIA to kill *Acinetobacter baumanii*, and *Lacticaseibacillus rhamnosus*. Pregnant women and others that may be immunocompromised are at particular risk should they be exposed to L. monocytogenes. These bacterial agents will be propagated as described previously using BSL2 containment. They will also utilize siRNA to silence NOTCH-1 and PLA2-IIA expression in oral epithelial cells that have been exposed to bacterial pathogens. The siRNA is obtained from Santa Cruz Biotechnology. Gene silencing is confirmed by FACS or qRT-PCR. Dr. Gonzalez laboratory will also co-culture human oral epithelial cells with oral commensal bacteria (*Streptococcus gordonii*, *Streptococcus sanguinis*, *Veillonella parvula*, *Actinomyces naeslundii*, *Capnocytophaga sputigena*, and *Neisseria mucosa*) to examine transcriptional and translational responses to these bacterial agents. *Fusobacterium nucleatum* is utilized as a control in these experiments. They will examine the mRNA and protein levels for 8 specific chemokines. They will also utilize qPCR to evaluate the expression of 4 specific miRNAs in these cells. All bacterial agents in this project will be handled at BSL2 containment as previously described. In Project 3, Dr. Gonzalez laboratory seeks to explore whether oral bacteria can activate HIV in latently infected macrophages. The THP-1 cell line will be challenged with *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Streptococcus mutans*, and *Candida albicans*. Cells will be harvested via lysis to determine HIV promoter activation via luciferase activity. Cells will also be subjected to flow cytometry in the UK Flow Cytometry Core facility and cytokine measurements via ELISA. Due to the increased risk of working with a cell line latently infected with HIV, these procedures will be completed at BSL2+ containment. All work will take place in a BSC until potential biohazards are inactivated via lysis or fixation. This work purposefully does not involve the use of sharps, needles, blades, etc., significantly reducing the risk of exposure via needlestick. Cell samples will be centrifuged in a bucket with safety-lid. Additional signage will be posted to indicate ongoing work with HIV. Lastly, Dr. Gonzalez laboratory seeks to examine the sex-related effects of aging in gingival health and periodontitis. This project will utilize young mice to obtain plaque samples to quantify estrogen receptor expression. This last project does not involve the use of any biohazardous materials.

IBC Discussion & Vote:

The protocol IBC-25-70 (version 12.0) was approved pending minor modifications as listed below:
*
ANIMAL RESEARCH – Animals with Biohazards table: The route of administration for *P. gingivalis* is currently listed as “gavage.” Please update the table to more specifically list “oral cavity gavage.”
SCIENTIFIC SUMMARY:

1. Please add more description of biosafety considerations for the following procedures that could possibly generate aerosols:
    a. Removing samples from centrifuge tubes, and,
    b. Washing multiwell plates.
2. Clearly identify what procedures will involve the use of Sharps
3. Please spell out acronyms being used, specifically SPLA-2A.
4. Include a description of signs and symptoms of accidental exposures to the agents in use. Include the specific risks to pregnant women and fetuses. Please clearly state that laboratory personnel are trained to recognize these signs/symptoms of potential exposure and next steps in case of exposure.
5. Please describe whether flow cytometry being performed on fixed or unfixed cells.
6. Include a description of the disinfection steps for cell culture supernatants and washes as these materials from HIV-infected cell lines may still be infectious.

*

Micheal Mendenhall initiated the motion. Carol Pickett seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

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PI: Christopher Fry

IBC Protocol Number: IBC-25-74
Protocol Title: Cellular regulators of skeletal muscle plasticity
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: NIH Guidelines Section IV-B-7, UK Administrative Regulation 6.3, UK Administrative Regulation 6.9, OSHA Act of 1970 Clause 5(a)(1), OSHA 29 CFR 1910.1030, NIH Guidelines Section III-D-1, NIH Guidelines Section III-F, NIH Guidelines Section III-F-1
Maximum Containment Level: Biological Safety Level 2 - Enhanced (BSL2+), Animal Biological Safety Level 1 (ABSL1)
Primary Reviewers: C. Haughton, J. Smalle, M. Mendenhall

Brief Project Overview:

The goal of our research is to understand the cellular and molecular mechanisms that allow striated skeletal muscle to adapt to external stimuli (i.e. atrophy and hypertrophy). We study cells, animals and human subjects to better understand how muscle adapts when challenged to develop interventions to improve muscle health and function in conditions where it is compromised, like weakness and frailty associated with illness and aging.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Cell culture, DNA/RNA isolation/purification, Histology, Imaging/Microscopy, Immunohistochemistry, PCR/qRT-PCR, Transfection, Use of Human Source Material(s), Use of viral vectors
Transport: Yes
Materials Transported: Biohazardous Materials, Animals
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: Follistatin/mouse /cell growth/housekeeping/expression/Mouse/plasmid; Cre recombinase/bacteriophage P1/recombinase/expression/mouse cells/Ad5CMV; GFP/jellyfish/tracking/expression/mouse /Ad5CMV; GDF8 (myostatin)/mouse/skeletal muscle cell regulation/silencing/mouse cells/siRNA/miRNA; Histone deacetylase 4 (HDAC4)/mouse/skeletal muscle cell regulation/skeletal muscle cell regulation/skeletal muscle cell regulation/plasmid; green fluorescent protein (GFP)/jellyfish/tracking/expression/Mouse/plasmid
Vector(s) [Vector Category/Vector Technical Name]: Plasmid/Fst (NM_008046) Mouse Tagged ORF Clone; Adenovirus/Ad5CMVCre-eGFP; Plasmid/HDAC4 (NM_207225) Mouse tagged ORF Clne; Plasmid/GFP Mouse tagged ORF clone; Naked nucleic acid/Mstn Mouse siRNA Oligo Duplex (Locus ID 17700); Naked nucleic acid/siRNA Related Product, Trilencer-27 Universal scrambled negative control siRNA duplex, SKU SR30004
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Animal/Myoblast; Animal/Fibroblast; Human/Myoblast; Human/Fibroblast; Human/MRC-5 fibroblast; Animal/C2C12

Animal Use: Yes
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Plasmid/electroporation/isoflurane anesthesia/ABSL1/gloves,labcoat/ABSL1/No/N/A

Risk Assessment/Discussion:

Dr. Fry has submitted a renewal of his existing IBC protocol entitled *Cellular regulators of skeletal muscle plasticity*. Dr. Fry's current IBC protocol will expire on July 13, 2025. In this renewal, Dr. Fry's laboratory seeks to understand the cellular and molecular mechanisms that allow striated muscle to adapt to external stimuli using in vitro and in vivo models. Dr. Fry's laboratory will receive human and mouse skeletal muscle tissue samples and utilize primary human and mouse cells to isolate DNA and RNA to evaluate the expression of specific genes and histone modification in muscle cells and tissue. They will also obtain human blood samples from the UK CCTS facility. Biohazardous samples are transported to the Fry laboratory in closed, sealed, shatter-proof containers. Bone graft aspirate will also be obtained from UK Healthcare for fixation and cell sorting in the UK FACS facility. For all work with human source materials, lab personnel will wear lab coat or gown, disposable gloves, and eye protection. Human samples will be manipulated within the BSC located in Dr. Fry's laboratory and centrifuged using sealed buckets/cups that are loaded/unloaded within the BSC and wiped with disinfectant prior to removal. Dr. Fry's animal work seeks to over-express Follistatin in mouse skeletal muscle following joint injury to determine if follistatin expression rescues atrophy and weakness. To do this, mice will be anesthetized and administered plasmid DNA via injection into the quadriceps muscle. Immediately following injection, quadriceps muscle will be subjected to electroporation. HDAC4 under the cytomegalovirus promoter with a GFP tag and control plasmid only expressing GFP will be utilized. Mice will be allowed to recover and euthanized at various timepoints to harvest quadriceps muscle for paraformaldehyde fixation and immunohistochemistry. Dr. Fry's laboratory will also utilize siRNA to knock down expression of GDF8 and Myostatin. siRNA is obtained from Origene. Sharps are not utilized during siRNA work, significantly minimizing the risk of accidental parenteral inoculation. Lab personnel will wear gloves, lab coat, and protective eye wear for siRNA work. The siRNAs obtained from Origene are designed to target mouse-specific sequences. Dr. Fry's laboratory also intends to utilize adenovirus vector obtained from the University of Iowa Gene Transfer Core facility in order to induce the depletion of N0WASp in satellite cells. Skeletal muscle tissue from floxed N-WASp mice is harvested and transduced with Adeno-Cre GFP or GFP empty vector. Dr. Fry's laboratory will not be packaging adenovirus. This is done by the University of Iowa Gene Transfer Core facility. Work with adenovirus will be completed within a BSC, and personnel will wear double-gloves, protective eyewear, and disposable lab coat. Adeno-transduced cells are harvested within 72 hours for immunohistochemistry, RNA and protein extraction. Liquid cell culture waste will be treated with household bleach (10% final concentration) prior to disposal. Adeno-transduced cells are washed a minimum of 3-times prior to any downstream experiments. While the adenovirus transgenes (Cre and GFP) are not particularly hazardous, even replication-deficient adenovirus is capable of causing eye damage if exposed. As such, all work with adenovirus and adenovirus-transduced cells will be performed inside a BSC. Materials are not removed from the BSC until fixation for imaging or other lysis steps that will inactivate any biohazards present. Cell culture and work with adenovirus is performed in dedicated spaces at BSL2+ containment. All animal work will be completed at ABSL1 containment using ABSL1 housing.

IBC Discussion & Vote:

The protocol IBC-25-74 (version 8.0) was approved pending minor modifications as listed below:

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SCIENTIFIC SUMMARY:

1. Typographical error - After the first paragraph, the first line item under "Risk mitigation…" states, "…spillage of the aspirate will be accomplished via…" Rewording the phrase to, "…will be avoided via…"
2. Please clarify whether all plasmid materials in use are obtained from a commercial source or if these will be amplified in the lab. If the latter, please add a description of how this is performed, including risk mitigation and waste management.
3. Include more information about how exposure risks are minimized including how samples are being removed from tubes after centrifugation.

*

Michael Mendenhall initiated the motion. Jan Smalle seconded the motion. All IBC members present (13) voted in favor of the motion.

*

Conflicts of Interest: None

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PI: Hong Lu

IBC Protocol Number: IBC-25-79
Protocol Title: Mechanisms of Aortic Aneurysm and Atherosclerosis
Protocol Type: Renewal
Amendment To: N/A
Applicable Guidelines & Regulations: UK Administrative Regulation 6.9, NIH Guidelines Section IV-B-7, OSHA Act of 1970 Clause 5(a)(1), NIH Guidelines Section III-F-4, NIH Guidelines Section III-D-4, OSHA 29 CFR 1910.1030, NIH Guidelines Section III-F, NIH Guidelines Section III-F-1, UK Administrative Regulation 6.3, NIH Guidelines Section III-E-1
Maximum Containment Level: Biological Safety Level 2 (BSL2), Animal Biological Safety Level 1 (ABSL1)
Primary Reviewers: C. Haughton, X. Li, T. Chambers

Brief Project Overview:

Background and Objectives: Cardiovascular diseases such as atherosclerosis, aortic aneurysms, and aortic dissection develop asymptomatically over time until a devastating event occurs, such as thrombus formation, occlusion, or rupture. To study the mechanisms of atherosclerosis, we use mouse models with hypercholesterolemia. To study mechanisms of aortic aneurysms and dissections, we use chemical or genetic manipulations: (1) angiotensin II infusion, (2) BAPN in drinking water (BAPN is 3- aminopropionitrile fumarate), (3) combined administration of AngII and BAPN, and (4) genetic manipulations.

Summary of Biohazard Materials & Manipulations:

Manipulations Planned: Animal work (breeding, surgeries, etc.), Bacterial culture, Cell culture, DNA/RNA isolation/purification, Histology, Genetics, Use of Human Source Material(s), Immunohistochemistry, PCR/qRT-PCR, Transformation, Transfection, Use of viral vectors, Imaging/Microscopy, Flow cytometry/Cell sorting, Proteomics

Transport: Yes
Materials Transported: Animals, Biohazardous Materials
Infectious Agent(s)/Natural Host(s): Human Sourced Materials (RG2-cells, tissues, bodily fluids, organs, etc.)/Human; Non-Human Primate (NHP) Materials (RG2)/Cynomolgus Monkey
Source & Nature of Inserted Nucleic Acid(s) [Gene Information/Gene Source/Gene Category/Use of Construct/Host(s)/Vector(s)]: angiotensinogen (AGT) /mus musculus C57BL/6 /regulatory gene /knockout or expression /mouse/AAV/; LRP1/mus musculus C57BL/6 /membrane protein /knockout /mouse/ASO/; LRP2 (megalin)/mus musculus C57BL/6 /membrane protein /knockout /mouse/ASO/; ASO of LRP1/synthetic /membrane protein /knockout /mouse/injections /; ASO of LRP2/synthetic /membrane protein /knockout /mouse/injections /; ASO of PCSK9/mouse/regulatory gene/knockout/mouse/Injections/; ASO of cubilin /synthetic /membrane protein /knockout /mouse/injections /; ASO of MMP9/synthetic /enzymatic protein /knockout /mouse/injections /; GFP/N/A/tracking gene /expression /mouse or cells /genetic or expression /; RFP/N/A/tracking gene /expression /mouse or cells /genetic or expression /; PCSK9/mouse/Cholesterol regulation /expression /mouse/AAV/; ASO of AGT and GalNAc AGT/synthetic /regulatory gene /knockdown /mouse/injections /; ASO of PAI-1/synthetic /regulatory gene /knockdown /mouse/injections /; ASO of SR-BI/synthetic /regulatory gene /knockdown /mouse/injections /; ASO of LDLR/synthetic /regulatory gene /knockdown /mouse/injections /; ASO of ApoC3/synthetic /regulatory gene /knockdown /mouse/injections; ASO of ANGPTL3 /mouse/regulatory gene/knockdown/mouse/Injections
Vector(s) [Vector Category/Vector Technical Name]: Naked nucleic acid/ASO (antisense oligonucleotides); Adeno-assocaited Virus (AAV)/AAV
Cell line(s) Used [Cell Line Type/Cell Line Technical Name]: Human/umbilical cord vein endothelial cells (not from human tissue samples); Human/HL-60; Human/THP-1; Human/HEK 293; Human/Human Fibroblasts; Animal/Cos 7; Animal/Peritoneal macrophage; Animal/endothelial cells; Animal/smooth muscle cells; Animal/J774; Animal/RAW264.7; Animal/IC-21; Animal/CHO-K1; Animal/P388D1; Animal/WEHI-3; Animal/hepatocytes; Animal/embryonic fibroblasts; Animal/mouse proximal tubule cells; Animal/3T3-L1; Animal/F9; Animal/immortalized renal proximal tubule cells
Animal Use: Yes
Materials introduced into Animals [Animal Host Species/Biohazardous Material/Route of Administration/Restraint/Animal Experimental Procedures/PPE/Animal Housing/Agent Shedding/Special Practices & Procedures]: Mouse/Naked Nucleic Acid-r/sDNA, subcutaneous injection/Isoflurane Anesthesia (or None, see Special Practices/Procedures)/ABSL1/Yes/ABSL1/No/To minimize accidental injection or needle stick, anesthesia such as isoflurane will be used to restrain mice, or alternatively, Bite-resistant gloves should be worn if anesthesia is considered to have the potential to affect the pharmacokinetics of the reagents; Mouse/Viral Vector - Adeno-Associated Virus (AAV)/Intraperitoneal/Isoflurane Anesthesia (or None, see Special Practices/Procedures)/ABSL1/Yes/ABSL1/No/To minimize accidental injection or needle stick, anesthesia such as isoflurane will be used to restrain mice, or alternatively, Bite-resistant gloves should be worn if anesthesia is considered to have the potential to affect the pharmacokinetics of the reagents.

Risk Assessment/Discussion:

Dr. Lu has submitted a renewal of her existing IBC protocol entitled *Mechanisms of Aortic Aneurysm and Atherosclerosis*. The Lu Lab studies cardiovascular diseases such as atherosclerosis, aortic aneurysms, and aortic dissection. They utilize mouse models with hyperchloestereolemia to study atherosclerosis and utilize genetic and chemical manipulations to study aortic aneurysms and dissections. They will administer antisense oligonucleotides (ASOs) targeting mouse AGT, LRP1, LRP2, cublilin, PCKS9, MMP9, PAI-1, LDLR, ApoC3, ANGPTL3 and SR-BI via subcutaneous injection. ASOs are obtained from Ionis. They will also utilize adeno-associated virus expressing mouse PCSK9D377Y (a gain-of-function mutation in mouse PCSK9 protein) to induce hypercholesterolemia OR WT or mutant AGT to determine to role of conserved sequences in AGT function. AAVs are obtained from a commercial vendor ready for use. AAV will be administered to mice via intraperitoneal injection. ASOs and AAVs will be administered to mice using ABSL1 containment, and subsequently animals will be housed at ABSL1 containment. At the conclusion of this experiment, mice will be euthanized and tissues obtained to measure mRNA and protein of the target genes in mouse tissues. In another project, Dr. Lu's lab will obtain human tissues from the Pathology Core Laboratories at UK or from collaborators at the Baylor College of Medicine. Some human materials received will be fixed in 4% paraformaldehyde or 10% formalin upon receipt. Live human tissues received will be handled at BSL2 containment wearing lab coat, gloves, eye protection, surgical mask, or face shield. In another project, Dr. Lu's laboratory will utilize primary and immortal human or mouse cell lines to modify knock down expression of LRP1, LRP2, or AGT via siRNA or ASOs. SiRNAs and ASOs will be obtained from Ionis. This work will be completed at BSL2 containment with the PPE same PPE described for the previous project. Cells treated with siRNA or ASOs will be fixed and stained to visualize LRP1, LPR2, or AGT. Alternatively, RNA and protein will be isolated to quantify mRNA and protein via ELISA or Western blots. Lastly, Dr. Lu's laboratory will obtain tissue and plasma/serum from cynomolgus monkeys from Dr. Ryan Temel. Dr. Lu's laboratory will not work with any live NHPs. They will receive both fresh-frozen tissue and fixed, paraffin-embedded tissues from Dr. Temel. Work with NHP materials presents the potential for exposure to Herpes B virus, a zoonotic disease of NHPs that can be fatal in humans. NHP tissues in this project are not known to be infected with Herpes B, but it is possible that NHPs are harboring Herpes B infection. PPE will include eye protection, facial mask, gown or lab coat, and gloves. The primary risk of exposure of NHP materials is splash to mucous membranes or broken skin. Work with NHP materials will be completed at BSL2 containment. Dr. Lu's laboratory has 3 BSCs available in a dedicated cell culture room for work with NHP materials and human cell culture.

IBC Discussion & Vote:

The protocol IBC-25-79 (version 14.0) was approved pending minor modifications as listed below:

*

ANIMAL RESEARCH – Animals with Biohazards Table: Please update the administration routes to match the associated IACUCs: AAVs are administered IP, IV, and Subcutaneous; ASOs are administered IP and Subcutaneous.

SCIENTIFIC SUMMARY:

1. Please expand on what is being done with human and NHP samples that are obtained from collaborators. What analyses and downstream assays are performed with these samples?
2. Flow cytometry is indicated as a manipulation planned in the General Information tab but is not described in the Scientific Summary. Please describe experiments involving flow cytometry.

3. Frozen tissues embedded in OCT are noted to be fixed with acetone for inactivation. Is this method of fixation suitable for inactivation of potential pathogens?

Thomas Chambers initiated the motion. Xiangan Li seconded the motion. All IBC members present (13) voted in favor of the motion.
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Conflicts of Interest: None
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UK Biosafety Manual Chapter Review

UK Biosafety Manual - (Addition) Decon Considerations for specific equipment.docx
UK Biosafety Manual - Blenders, Mixers, Sonicators, Vortexers, Homogenizers.docx
UK Biosafety Manual - Flow Cytometers & Cell Sorters.docx
UK Biosafety Manual - Growth Chambers.docx
UK Biosafety Manual - Incident Reporting.docx
UK Biosafety Manual - Incubators.docx
UK Biosafety Manual - Institutional Biosafety Committee.docx
UK Biosafety Manual - Plant Biosafety.docx
UK Biosafety Manual - Poliovirus Infectious Materials.docx
UK Biosafety Manual - Microtome-Crytostat-Microstat.docx
UK Biosafety Manual - Laboratory Exit-Closing.docx
UK Biosafety Manual - Laboratory Furniture.docx
UK Biosafety Manual - Select Agents & Toxins.docx

The IBC had the following comments on submitted chapters:

1. Flow Cytometers & Cell Sorters: In the second paragraph, regarding the use of biological safety cabinets to house the flow cytometers, is there an alternative?
2. Blenders, Mixers, Sonicators, Vortexers, Homogenizers: The text reads that after the process is done, wait to open for one minute to allow aerosols to settle. This should be corrected to 30 minutes unless opened within a Biological Safety Cabinet (BSC) or other containment device suitable for containing biohazardous aerosols.

*
Biosafety Manual text will be updated by BSO for review at the next scheduled IBC meeting.

Incident Review

Nothing to report.

Protocol Issued Registration Numbers

Protocols issues registration numbers, including minor amendments. These protocols are exempt from IBC review and are registered with the UK Biological Safety Officer (BSO).

Papazoglou, Ioannis, Neuronal response to hypoglycemia, Amendment, BSO, IBC-24-35 (v.30.0), 7/2/2025
Kern, Philip, Inflammation in & Regulation of Obesity, Amendment, BSO, IBC-24-325 (v.17.0), 7/2/2025

Chattopadhyay, Saurabh, Innate immune responses to virus infection, Amendment, BSO, IBC-24-108 (v.41.0), 7/1/2025
Weisleder, Noah, Modifying gene expression in vitro and in vivo, Amendment, BSO, IBC-24-499 (v.44.0), 7/1/2025
Wolf, Katerina, Genetic manipulation and phenotypical analyses of Chlamydia trachomatis, C. muridarum, and C. pneumoniae, Amendment, BSO, IBC-24-51 (v.31.0), 6/26/2025
Tian, Changhai, B21-3788-M11: Intra- and Inter-organ communications by extracellular vesicles in the pathogenesis of cardiovascular and neurodegenertive disorders, Amendment, BSO, IBC-24-402 (v.22.0), 6/24/2025
Czuba, Lindsay, Mechanisms of Regulation of Bile Acid Homeostasis, Amendment, BSO, IBC-24-306 (v.35.0), 6/24/2025
Monje, Paula, Isolation, culture and analysis of human peripheral nerve cells, Amendment, BSO, IBC-24-102 (v.41.0), 6/18/2025
Wood, Jeremy, Anticoagulant Properties of Protein S, Amendment, BSO, IBC-24-161 (v.23.0), 6/17/2025
Izumi, Tadahide, A core protocol of X-ray irradiation for UK researchers, Renewal, BSO, IBC-25-71 (v.8.0), 6/17/2025
Izumi, Tadahide, DNA base excision repair in mammalian cells, Amendment, BSO, IBC-24-405 (v.22.0), 6/17/2025
Givens Rassoolkhani, Brittany, Sustained-release anti-neoplastic agents for the treatment of endometrial cancer, Amendment, BSO, IBC-24-401 (v.18.0), 6/16/2025
Ubil, Eric, PTP1b Inhibition Restores the Innate Anti-tumor Response During Chemotherapy, New, BSO, IBC-24-446 (v.14.0), 6/13/2025
Cai, Weikang, Understanding astrocytes and microglia functions in neurological diseases., Amendment, BSO, IBC-24-408 (v.45.0), 6/11/2025

Protocols Meeting Registration Requirements

Protocols that have been approved by the IBC pending minor modifications that have met approval requirements.

Blackburn, Jessica, Identifying mechanisms of cancer progression using lentiviral infected human cell lines, Renewal, IBC, IBC-25-60 (v.12.0), 6/26/2025
Blackburn, Jessica, Generation and use of transgenic zebrafish to study human cancer, Renewal, IBC, IBC-25-61 (v.12.0), 6/26/2025
Goehring, Lutz, Equid herpesvirus epidemiology, transmission, pathophysiology, vaccinology, Amendment, IBC, IBC-25-02 (v.25.0), 6/24/2025
Turner, Jill, Pharmacogenetics of Nicotine Dependence, Renewal, IBC, IBC-25-62 (v.13.0), 6/17/2025
Barrett, Terrence, B22-4064-M: Regulation of Intestinal Stem Cell Activation in Colitis, Amendment, IBC, IBC-24-326 (v.17.0), 6/13/2025
Mishra, Ila, Small peptide hormones in metabolic disorders, Amendment, IBC, IBC-24-89 (v.30.0), 6/12/2025

Dutch, Rebecca, B24-4323: Paramyxovirus and pneumovirus protein function, and HPIV3 polymerase and mRNA capping studies, Amendment, IBC, IBC-24-134 (v.20.0), 6/11/2025
Stewart, Andrew, Gene Therapy Approaches to Induce and Control Neuronal Growth in Rodents With Spinal Cord Injuries, Amendment, IBC, IBC-24-333 (v.63.0), 6/11/2025
Van Sanford, David, B22-3920: Accelerating the Development of FHB-Resistant Soft Red Winter Wheat Varieties, Renewal, IBC, IBC-25-51 (v.10.0), 6/6/2025
Temel, Ryan, Knockdown and overexpression of genes in mice and nonhuman primates using antisense oligonucleotides (ASO), Amendment, IBC, IBC-24-119 (v.18.0), 6/6/2025

IBC Training

Delena Mazzetti reminded IBC members that IBC Member training is available in the SciShield Course Directory (https://uky.scishield.com/raft/training/courses) and that all IBC members must complete IBC member training annually.

Adjournment

Doug Harrison initiated a motion to adjourn the meeting at 1:46pm. Michael Mendenhall seconded the motion. All IBC members present (13) voted in favor of the motion.