**THE UNIVERSITY OF WESTERN ONTARIO**
**BIOHAZARDOUS AGENTS REGISTRY FORM**
**Approved Biohazards Subcommittee: September 25, 2009**
**Biosafety Website: www.uwo.ca/humanresources/biosafety/**

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

| | |
|---|---|
| PRINCIPAL INVESTIGATOR | Anthony Jevnikar |
| SIGNATURE | |
| DEPARTMENT | Medicine |
| ADDRESS | A10-112, UH |
| PHONE NUMBER | 33688 |
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| EMAIL | jevnikar@uwo.ca |

Location of experimental work to be carried out: Building(s) Robarts Institute Room(s) 2298

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): Regulation of Tissue Injury and Novel Approaches to Tolerance Induction to Promote Long Term Renal Transplant Survival

**PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.**

Names of all personnel working under Principal Investigators supervision in this location:

| | |
|---|---|
| Ziqin Yin | ZhuXu Zhang |
| Xuyan Huang | Laura Baker |
| Shuang Wang | |
| Kelvin Shek | |
| Arthur lau | |

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

**1.0 Microorganisms**

1.1 Does your work involve the use of biological agents? ⊗ YES O NO
(including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ⊗ NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? E.Coli DH5 alpha
Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

1.2 Please complete the table below:

| Name of Biological agent(s)* | Is it known to be a human pathogen? YES/NO | Is it known to be an animal pathogen? YES/NO | Is it known to be a zoonotic agent? YES/NO | Maximum quantity to be cultured at one time? (in Litres) | Source/ Supplier | PHAC or CFIA Containment Level |
|---|---|---|---|---|---|---|
| DH5 | O Yes ⊗ No | O Yes ⊗ No | O Yes ⊗ No | 0.5 | Invitrogen | ⊗ 1 O 2 O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 3 |

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

**2.0 Cell Culture**

2.1 Does your work involve the use of cell cultures? ⊗ YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

| Cell Type | Is this cell type used in your work? | Source of Primary Cell Culture Tissue | AUS Protocol Number |
|---|---|---|---|
| Human | ⊗ Yes O No | kidney cell line | Not applicable |
| Rodent | ⊗ Yes O No | mouse kidney/spleen | 2006-069 |
| Non-human primate | O Yes ⊗ No | | |
| Other (specify) | O Yes ⊗ No | | |

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in:

| Cell Type | Is this cell type used in your work? | Specific cell line(s)* | Supplier / Source |
|---|---|---|---|
| Human | ⊗ Yes O No | PT-2 TEC | Philip Acott, Dalhousie university |
| Rodent | ⊗ Yes O No | NG1.1, CS3 and KO2S. | self developed |
| Non-human primate | O Yes ⊗ No | | |
| Other (specify) | O Yes ⊗ No | | |

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ⊗ 1 O 2 O 3

### 3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ⊗ YES O NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

| **Human Source Material** | **Source/Supplier /Company Name** | **Is Human Source Material Infected With An Infectious Agent? YES/NO** | **Name of Infectious Agent (If applicable)** | **PHAC or CFIA Containment Level (Select one)** |
|---|---|---|---|---|
| Human Blood (whole) or other Body Fluid | | O Yes ⊗ No O Unknown | | O 1 O 2 O 3 |
| Human Blood (fraction) or other Body Fluid | | O Yes ⊗ No O Unknown | | O 1 O 2 O 3 |
| Human Organs or Tissues (unpreserved) | kidney/Dalhousie university | O Yes ⊗ No O Unknown | | O 1 ⊗ 2 O 3 |
| Human Organs or Tissues (preserved) | | Not Applicable | | Not Applicable |

cell line PT-2 TEC

### 4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES ⊗ NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

| Bacteria Used for Cloning * | Plasmid(s) * | Source of Plasmid | Gene Transfected | Describe the change that results |
|---|---|---|---|---|
| | | | | |

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below O NO

| Virus Used for Vector Construction | Vector(s) * | Source of Vector | Gene(s) Transduced | Describe the change that results |
|---|---|---|---|---|
| | | | | |

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

4.7 Will this be expected to increase the containment level required? O YES O NO

**5.0 Human Gene Therapy Trials**

5.1 Will human clinical trials be conducted involving a biological agent? O YES ⊘ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

**6.0 Animal Experiments**

6.1 Will live animals be used? ⊘ YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used mouse

6.3 AUS protocol # 2006-069

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ ⊘ NO

6.5 Will the agent(s) be shed by the animal: O YES ⊘ NO, please justify:
not use in animal

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## 7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES ⊘ NO
- Pound source cats O YES ⊘ NO
- Cattle, sheep or goats O YES ⊘ NO
- Non-human primates O YES, please specify species _____ ⊘ NO
- Wild caught animals O YES, please specify species & colony # _____ ⊘ NO
- Birds O YES ⊘ NO
- Others (wild or domestic) O YES, please specify _____ ⊘ NO

## 8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ⊘ NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the $LD_{50}$ (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide detais:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

## 9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊘ NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

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**10.0 Plants Requiring CFIA Permits**

10.1 Do you use plants that require a permit from the CFIA? O YES ⊗ NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

**11.0 Import Requirements**

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 12.0 ⊗ NO

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

**12.0 Training Requirements for Personnel Named on Form**

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

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**13.0 Containment Levels**

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 Q 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus BIO-RRI-0018

O NO, please certify

O NOT REQUIRED for Level 1 containment

**14.0 Procedures to be Followed**

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: Nov 4/09

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.

_____

_____

_____

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

_____

_____

**15.0 Approvals**

UWO Biohazard Subcommittee: SIGNATURE: _____

Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____

Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____

Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

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# Description of PT-2 TEC

Renal proximal tubular epithelial cells play a crucial role in renal function.

Human PT-2 (proximal tubular cell) is a proximal tubular cell line derived from patient's urine samples and spontaneously transformed. Cells are not from a commercial seller, but a gift from Dr. Phillip Acott in Halifax and documented by the MTA sheet. The biohazardous material safety data sheet of PT-2 cells is not available from the lab of Dr. Phillip Actt. PT-2 Cells are cultured by K1+/+ medium with growth factor and serum. The cells express tubular epithelial cells marker, like CS13, CD26 as well as e-Cadherin. Cells will be used to test the role of IL-2 receptor and apoptosis in human kidney in vitro.

Dr. Anthony Jevnikar's lab
Robarts room 2298
Phone: 24204

REGULATION OF RENAL TUBULAR EPITHELIAL CELL INJURY TO PROMOTE KIDNEY ALLOGRAFT SURVIVAL



New Investigator, Clinician Scientist candidates and grant applicants should summarize the objective(s), hypothesis and research plan. Investigator and Senior investigator candidates should summarize their 5 year plan. Must include project title. (maximum one page)

**Regulation of renal tubular epithelial cell injury to promote kidney renal allograft survival**

Tubular epithelial cells (TEC) comprise more than 75% of renal parenchymal cells and their susceptibility to injury directly influences the long term function of the allograft. Post transplant, TEC death by apoptosis occurs with ischemia, reactive oxidative metabolites (ROM), nitric oxide (NO), pro-inflammatory cytokines (TNFα, IL-1) and cytotoxic T cells (FasL/Fas, Perforin/Granzyme B). The direct invasion of tubules (tubulitis) by mononuclear cells in fact is the primary feature of acute cellular rejection. We have previously made the novel observation that Fas mediated apoptosis of TEC is regulated by an endogenous inhibitor of caspase-8 activation (c-FLIP). Augmenting TEC capacity to resist injury might therefore be a useful strategy in renal transplantation but requires further understanding of complex pathways in TEC apoptosis. We have now found that TEC participate in Fas/FasL dependent apoptotic self injury ("fratricide") which contributes to allograft injury and that c-FLIP also regulates non-Fas pathways of TEC apoptosis. The regulation of Fas/FasL and c-FLIP in TEC may therefore occupy a position of central importance in diverse forms of transplant injury including "fratricide", nitric oxide, inflammatory cytokines and cytotoxic T cells. We hypothesize that augmenting TEC resistance to apoptosis by c-FLIP, Fas/FasL blockade and other approaches can promote allograft survival and prevent premature graft loss. The specific aims are:

**1.** To test if endogenous resistance of TEC to apoptosis and self injury occurs through compartmentalization of Fas/FasL and by regulation of c-FLIP. We will use primary culture and immortalized TEC (CS3.7, MRM-*gld*, M3.1-*lpr*) we have created and confocal microscopy, to define the role of apical/basolateral compartmentalization of Fas and FasL as a mechanism of resistance to self apoptosis, and the effect of NO and pro-inflammatory cytokines on disrupting TEC polarity and loss of normal sequestration of FasL/Fas. By over-expression, we will test whether c-FLIP can maintain TEC integrity and prevent Fas/FasL, TNFα and nitric oxide (NO) apoptosis. We will test the capacity of small interfering RNAs (siRNA) to target Fas, FasL or caspase-8 to abrogate TEC apoptosis *in vitro*. We use both RT-PCR and Western blot analyses for expression studies and will confirm TEC apoptosis in response to anti-Fas, cytokines and cytotoxic allogeneic effector CD4 and CD8 T cells in TEC-lymphocyte co-cultures using several apoptosis methodologies.

**2.** To test the effect of attenuating TEC apoptosis on transplant function *in vivo*. We will create a transgenic mouse in which androgen inducible c-FLIP expression is specifically targeted to TEC in kidneys for use as transplant donors. The ability of siRNA targeting Fas, FasL and caspase-8 to reduce kidney injury in a transplant model will also be tested using *in vivo* and *ex vivo* donor pre-treatment.

**3.** To assess the role of endogenous NO within donor tissue in graft function and survival. TEC from iNOS null mice will be tested for resistance to apoptosis and self injury following cytokines. iNOS null kidneys will transplanted to assess effects on TEC survival as well as long term graft function.

**4.** To test if indeolamine 2,3-dioxygenase (IDO) augments Fas mediated TEC apoptosis. IDO is an enzyme which is involved in fetal and T cell tolerance by increased susceptibility of T cells to Fas mediated apoptosis. We have made the observation that TEC express IDO which may contribute to TEC fratricide. We will silence IDO in TEC with siRNA and test whether IDO augments FasL mediated apoptosis of TEC.

The regulation of apoptosis in TEC represents a critical component of parenchymal cell defense during inflammation. The resistance or susceptibility of TEC to apoptosis may define long term allograft function. Our original observation of FLIP expression in TEC and novel demonstration of Fas dependent TEC fratricide, as well as our capacity to perform mouse kidney transplants will allow us to answer the questions we have raised. This proposal outlines important directions in the protection of TEC, which we hope will translate to truly new therapies for transplant patients.

OP GRANT 2005-2010