THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological 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 biological agents 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 Biohazards 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	**Dr. Anthony M. Jevnikar**
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Location of experimental work to be carried out: Building(s) WVB and SDRI Room(s) Non-Human Primate Unit and SDRI Room 121

*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 15.0, Approvals).

FUNDING AGENCY/AGENCIES: National Institute of Health
GRANT TITLE(S): Induction of Renal Allograft Tolerance in Monkeys with anti-CD45Rb and anti-CD40 Based Therapies

List all personnel working under Principal Investigators supervision in this location:

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Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

1. In addition to practicing the procedures and protocols described in the University of Western Ontario's **BIOSAFETY GUIDELINES AND PROCEDURES MANUAL For Containment Level 1 & 2 Laboratories** www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/biosafety_manual.pdf the following procedures will be adhered to, to ensure that the ***PHAC-Level 2+ biosafety requirements are met.***
2. The door to the fully enclosed Tissue Culture room (SDRI TCR-Level 2+) will remain shut at all times and will be locked when vacated (Re: Q.4, Q.19 and Q.34 of PHAC - Public Health Agency of Canada permit). The Non-Human Primate Unit (NHP) in the West Valley Building (WVB) doors will be locked at all times and entry can only be accessed with personal identification card and magnetic strip card reader.
3. Inward directional airflow will be checked and ensured by using a laboratory-based draft detector and lab staff, and is consistently monitored by UWO-Physical Plant.
4. Solid-front gowns (disposable) with tight-fitting wrists will be worn at all times and only within the TCR-Level 2+ within which the biosafety cabinet (BSC) is housed within SDRI, as well throughout the NHP unit of WVB. Disposable gloves and foot covers will also be worn at all times during manipulation of the NHP tissue within the BSC of SDRI and throughout NHP-WVB and will be removed upon exit of both facilities (Re: Q.20, Q.24 and Q.25).
5. Potentially infectious agents (frozen macaca lymphocytes, plasma and/or sera) are and will be stored in a locked liquid nitrogen dewars (-70°C) stored outside the containment area (in adjacent cold room) of SDRI. Similar samples will be stored in a Ultralow freezer (-80°C) located within the NHP-WVB unit. The leakproof dewar and Ultralow freezer are both well-labeled with signage indicating the biohazardous agents contained within (Re: Q.35).
6. All activities with potentially infectious macaca blood and tissue are conducted in a Biosafety cabinet (BSC) housed within SDRI-TCR-Level 2+ and/or in the Operating room of the NHP-WVB (Re: Q.36).
7. No activities with potentially infectious macaca blood and tissue will occur outside the BSC and therefore never will occur outside of SDRI-TCR-Level 2+ and thus justifies that only this room be designated as Level 2+ (Re: Q.37) within SDRI. In contrast within the NHP-WVB, activities with potentially infectious macaca blood and tissue, as well as interactions and manipulations of live animals will occur in the Operating room and animal housing rooms, outside the containment environment of BSCs. Thus the entire NHP-WVB must be designated as Level 2+.
8. Sealed safety centrifuge buckets (Allegra X-15R centrifuge/SW4750 swinging-bucket rotor; Beckman Coulter) will be loaded and unloaded with NHP samples only within the BSC of SDRI-TCR-Level 2+ (Re: Q.38).
9. Researchers in SDRI will not require disinfection of footwear and changing of clothes due to the use of disposable footcovers and solid-front gowns. An eyewash shower is housed within the outer laboratory (Level 2) of Room 121, as well as a full emergency body shower is available and functioning within the same SDRI hallway as our laboratory (Re: Q.130).
10. Animal care staff working in the NHP-WVB are well-trained and will follow SOPs set out by UWO-ACVS in conjunction with UWO-Biosafety regarding personal protection equipment use and removal, as well as emergency shower locations provided within the unit (Re: Q.130)
11. Written protocols specific to each project in progress involving the macaca tissues and blood are available and centrally-located within the outer laboratory of SDRI Room 121, as well as within the office of NHP-WVB. In addition, individual experiments/data are recorded daily in Dr. Jacqueline Arp's and Dr. Yuexia Ma's bound research data book in SDRI; while detailed records of all activities involving the NHP are maintained by the appointed on-call veterinary technicians in the NHP-WVB unit (Re: Q 138).
12. SDRI personnel (Dr. Jacqueline Arp and Dr. Yuexia Ma) possess a high degree of proficiency in microbiological and sterile practices and techniques. Similarly, staff involved in the NHP care and research occurring in the NHP-WVB (i.e. Dr. Patrick Luke, Dr. Ian Welch, Dr. Zhu Lan, Tracy Hill, Heather Cadieux, Tamie Fulford, Rachel Daniels, and part-time staff) are well-trained in handling, care and surgical procedures involving the NHP (Re: Q.140).
13. In case of emergency (i.e. fire in the lab), when there is no time to remove the outer layer of personal protection equipment (i.e. gloves, gowns and gloves), research staff will simply leave as they are and report to the incident commander (wearing orange vest).
14. All research involving mouse blood and tissues will following the guidelines for Level 2 Containment as described in the **UWO - BIOSAFETY GUIDELINES AND PROCEDURES MANUAL For Containment Level 1 & 2 Laboratories**, www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/biosafety_manual.pdf to ensure that the necessary PHAC-Level 2 requirements are met.
15. All biohazardous waste receptacles in SDRI-Room 121 and NHP-WVB are to be clearly labelled with Biohazard tape so that all parties, including caretaking, can unmistakably identify these containers. All biohazardous material for autoclaving is to be placed in an autoclave bag before leaving the lab. All such waste will be autoclaved and verified for decontamination as per current Western Autoclaving and Verification Standard Operating Procedures.
16. If more information is required, staff will either consult he website: www.uwo.ca/humanresources or contact the biosafety officer at extension 81135.

Please include a research summary or teaching protocol.

Induction of Renal Allograft Tolerance in Monkeys with anti-CD45RB and anti-CD40 Antibody-based Therapies

A. RATIONALE AND HYPOTHESIS:

Despite improved immunosuppression, long-term renal allograft survival has not lived up to predictions. Tolerance promises to improve long-term outcome by reducing both acute and chronic rejection and risks of chronic immunosuppression. Moreover, peripheral strategies avoid the risks of conditioning and GVHD associated with hematopoietic chimerism. The quest for peripheral tolerance in human transplantation remains unfulfilled and new insight is required. It has become clear that that therapies involving individual agents are unable to induce tolerance in non-human primates (NHP). Yet, individual agents that are ineffective when used alone, can markedly prolong graft survival when used in combination. These findings a provide logical foundation for the **central hypothesis of this proposal:** ***the complexity of immune responses in primates will require combination therapy to induce transplant tolerance using agents known to exhibit potent synergy in stringent murine allograft models.*** In this regard, we have shown that regimens combining α-CD45RB with agents that inhibit DC maturation or block CD40/CD40L interactions are exceedingly potent and can induce tolerance in the most stringent murine allograft models. Moreover, studies suggest that α-CD45RB acts through a unique mechanism that involves de novo induction of CTLA-4 and Foxp3 Tregs. This may explain potent synergy with agents that block DC maturation and also promote Tregs. Preliminary findings using an anti-human CD45RB as a single agent in monkey renal transplantation demonstrates remarkable long-term survival in some recipients. Based on potent synergy both in terms of tolerance induction and in terms of mechanisms of action, we now propose to combine α-CD45Rb with α-CD40. Mechanistic studies will be performed to extend insight gained from murine studies, and specifically address the effects of the added agents on Treg generation, anergy, deletion, and inhibition of B cell and DC activation.

B. BACKGROUND AND APPROACH:

1) Tolerance in clinical transplantation promises to improve long-term outcome by reducing acute and chronic rejection and risks of chronic immunosuppression. Moreover, peripheral strategies avoid risks of conditioning and graft-versus-host disease. Despite progress, peripheral tolerance in primates remains elusive and new agents with novel mechanisms of action are required for preclinical testing.

2) Anti-CD40 antibody mAb (α-CD40): While tolerance was not achieved, costimulatory blockade with α-CD154, the ligand of CD40 (CD40L) remarkably prolonged renal allograft survival in NHPs, with survival in some for >1 yr. after mAb therapy was discontinued (12). Thus blockade of CD154/CD40 interactions was remarkably potent and raised the possibility that addition of synergistic agents might tip the balance towards true tolerance. Unfortunately, CD154 is expressed on human platelets and clinical trials with 2 different α-CD154 mAbs were both stopped due to thromboembolic events (14). To inhibit of this key costimulatory pathway yet avoid thromboembolism, we wish to examine the efficacy of using α-human CD40 mAbs instead.

3) Anti-CD45RB mAb (α-CD45RB): The CD45 family of protein tyrosine phosphatases (PTPases) plays a critical role in T cell activation, in part through regulating src-family tyrosine kinases (PTKs) (26). α–CD45Rb mAb recognizes and inhibits the activation and proliferation of CD4 CD45RBHi T cells that would otherwise induce transplant rejection and/or autoimmune disease (30, 32).

C. OBJECTIVE AND SPECIFIC AIMS:

To test new therapeutic strategies for the induction of Non-Human Primate (NHP) transplant tolerance, we will carry out the following specific aims:

AIM 1: To determine if α-CD45RB synergizes with CD40 blockade to promote tolerance in NHP.
AIM 2: To determine if α-CD45RB synergizes with sCD83 to promote tolerance in NHP.
AIM 3: To Determine The Mechanisms By Which α-CD45RB-Based Combination Therapy Promote Tolerance in NHP.

We will perform sequential domino transplants using male Cynomologus monkeys where each animal serves as both a donor and recipient (e.g. transplant order would be: A to B, B to C, and C to A). Aims 1 and 2 will focus on strategies combining α-CD45RB with α-CD40, and with sCD83, respectively. The main focus of the first two aims is to study the outcome of the therapies, while defining measures of immune responsiveness mechanisms. Studies will center on what additional agents can contribute to anti-CD45RB therapy to promote engraftment. Aim 3 will determine what is required to prevent immunological break-through and identify novel pathways for the generation of tolerance.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES **XX** NO
(non-pathogenic and pathogenic biological agents 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 O NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
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
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 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? **XX** 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	O Yes **XX** No		Not applicable
Rodent	O Yes **XX** No		Not applicable
Non-human primate	**XX** Yes O No	Cynomolgus macaques – spleen, blood and lymph nodes; West Valley Building Non-Human Primate Unit	2007-118 renewal is currently under review
Other (specify)	O Yes **XX** No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes **XX** No		
Rodent	O Yes **XX** No		
Non-human primate	O Yes **XX** No		
Other (specify)	O Yes **XX** 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 O 1 O 2 **XX** 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES **XX** 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 O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

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 **XX** 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 from transformation or tranfection

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

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 from transduction

* 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 **XX** 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? **XX** YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used Cynomolgus macaques

6.3 AUS protocol **# 2007-118 renewal is currently under review**

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

6.5 Will the agent(s) be shed by the animal: **XX** YES O NO, please justify:

** there is the potential of shedding of zoonotic viruses such as Herpes B virus* (HAV-1)

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? **XX** YES O No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES **XX** 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 details:_____ O NO

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

9.0 Insects

9.1 Do you use insects? O YES **XX** 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 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? O YES **XX** 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 YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? **XX** YES, please give country of origin USA O NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? **XX** YES, *permit # on file at UWO purchasing* 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____ 

13.0 Containment Levels

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

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

XX YES, permit # if on-campus -- Level 2 Plus #BIO-UWO-0173
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 are provided up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

Signature

SIGNATURE _____ Date:_____

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

** No sharps will be used at SDRI at any during manipulation of macaca blood and tissue samples*

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

** Immediately address injured site following "Decontamination kit and protocol" housed in tissue culture room of SDRI 121 located above the incubator tower in clear tub. Insure that injured site is scrubbed vigorously enough to draw blood. After initial decontamination and cleansing of site, go to UWO-Staff Health or University Hospital Emergency immediately with injury report papers in hand.*

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE:_____
Date:_____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE:_____
Date:_____

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

Special Conditions of Approval: