

PROTOCOL FOR ANIMAL USE AND CARE*Handwritten forms are not accepted*

EH&S USE ONLY

PROTOCOL # 9840**EXPIRES:** _____

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone:	
After hrs.#:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus macaque	19/year	CRPRC

Project Title: **Development of a Lymphocyte Trafficking Model in Rhesus Macaques**

Overnight housing location::	CRPRC	Day use only :	CRPRC
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

Procedures: Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Rhesus macaques will be inoculated intranasally/intravenously with a pathogenic SIV or SHIV strain. Lymphocytes will be isolated from animals 7-28 days post infection and labelled with a cytoplasmic dye *ex vivo*. For one group, lymphocytes will be infected *in vitro* before autologous intravenous transfer. Cell migration will be assessed with biopsy and at necropsy.

Special Husbandry Requirements: Describe any special requirements your animals have with respect to **food, water, temperature, humidity, light cycles, caging type, bedding**, or any other conditions of husbandry.

CRPRC protocols of SIV-infected macaques in BSL-2 housing.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

Hazardous Materials (*only if in the animal room*):

Infectious Agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	Simian Immunodeficiency Virus (SIV), Simian Human Immunodeficiency Virus (SHIV)
Radioisotopes?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	UARP	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pcstillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

Background: AIDS is a fatal disease caused by infection with the human immunodeficiency virus type 1 (HIV-1). No vaccines for preventing HIV-1 infection and no effective therapies or cures for AIDS are available. An appropriate animal model for investigating mechanisms of HIV-1 infection and anti-viral immune responses is not available. Selected strains of simian immunodeficiency virus (SIV), a primate lentivirus genetically related to HIV, causes a fatal AIDS-like disease in macaques.

Hypothesis: The hypothesis is that migration of normal T lymphocyte populations from an SIV-uninfected animal differs from migration of both T lymphocyte subsets from animals infected with a pathogenic as well as a non-pathogenic viral strain. Furthermore, we postulate that migration patterns and tissue distribution in vaccinated animals will be distinct and might reveal mechanisms of protective immunity. Studies involving uninfected, and SIV-infected animals with a non-pathogenic and a pathogenic strain will allow for understanding dissemination mechanisms of both infected and uninfected lymphocytes upon intravenous transfer.

Objective: The objective of this project is to test T lymphocyte migration patterns in rhesus macaques, in both SIV-infected and uninfected animals. This project will utilize pathogenic and non-pathogenic SIV/SHIV strains to infect lymphocytes in vitro and in vivo and then assess lymphocyte trafficking. Macaques will be monitored for virus load, antiviral immune responses, and tissue distributions of labelled cells, both infected and uninfected. Trafficking patterns will be compared between healthy, SIV-uninfected and SIV-infected as well as vaccinated animals. If differences will be found, we will correlate these distinct migration and virus dissemination patterns with protective immunity and disease viral load data.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- | | | |
|---|---|--|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets: food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery surgical procedures | <input type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below) |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

A diagram of the five animal groups in this study are attached as Appendix. This protocol describes a research project that is pilot in nature. Therefore, we will initially start with groups of 2 animals per group, and then proceed with 2 more animals for Groups 1-4. Only for Group 5 are we unable to recruit 4 animals; three animals will be obtained from a currently ongoing study from a different investigator. These animals constitute a great resource for our particular project due to the animal's previous virus exposure and their specific immunologic properties (MHC genotype); these animals would be euthanized if not used for this study.

Group 1 will not be SIV-infected. Maximal bleeding volume not to exceed 20% of total blood volume and 12 ml/kg/month, will be drawn at onset of experiment for purification of lymphocytes, dye labeling and intravenous adoptive transfer within 24 hours of bleed, following standard CRPRC operating procedure. Animals in all groups will be treated identically from adoptive transfer set point.

Groups 2 and 3: Macaques will be inoculated once with 1.6-2 mls of virus (pathogenic and non-pathogenic SIV/SHIV strain respectively) in tissue culture fluid by dripping 0.3 mls into each nostril and 1 ml into the oral cavity following the CRPRC standard operating procedure (SOP). Only viruses which replicate in cultures of rhesus PBMC will be used as inoculum in macaques. After inoculation, 3-5 blood samples will be drawn between weeks 1 and 4 of inoculation, not exceeding a combined volume of 20 ml total for all 5 bleeds. At the peak viral load time point (between weeks 2 and 4), 10-20% of total blood volume will be drawn from the animals not to exceed the allowable total blood volume of 12ml/kg/month per animal.

Group 4: Animals will not be inoculated with SIV/SHIV by the mucosal route; animals will be bled maximal volume at onset of experiment (according to SOP see above). Lymphocytes will be infected with SIV/SHIV *in vitro* for 6-12 hours, before CFSE-dye labeling and adoptively transferring the cells back into the animals, thereby transferring SIV/SHIV-infected lymphocytes.

Group 5: 3 Genotyped animals (Major Histocompatibility Complex Class I allele Mamu-A*01) are available to us from another ongoing study. These animals have been vaccinated with a SHIV strain. The animals will be inoculated with a pathogenic SHIV strain similar to Groups 2 and 3 above by the same intranasal/oral route (0.3mls into each nostril, 1 ml into oral cavity). These animals will allow us to track recall responses and migration of particular antigen-specific T lymphocyte subsets and may allow us to track memory T lymphocyte migration and compare it to effector T lymphocytes. 3-5 bleeds will be obtained between week 0 and week 3 of inoculation, and maximal bleed will be obtained between weeks 1 and 4 for dye labeling and adoptive transfer.

Lymphocytes derived from animals in Groups 1-5 will be isolated on a ficoll gradient, and cells labeled *ex vivo* with cytoplasmic, viable dye carboxy fluorescein succinimidyl ester (CFSE) under sterile conditions (tissue culture laminar flow hood, sterile RPMI). This dye has been extensively used and tested in tissue culture as well as in many cell tracking and proliferation experiments *in vivo* in mice (see literature search). We will use this dye at the same concentrations (2.5-10 μ M) for labeling before washing out the remainder and transferring cells back into the animals. The cytoplasmic label allows tracking of cells and their proliferative responses *in vivo* for up to 8 weeks. Dye-labelled Lymphocytes will be autologously transferred by the intravenous route within 24 hours of isolation. Animals will be anesthetized once between days 3-10 post transfer with ketamine and Isoflorane according to Standard Operating Procedures (SOP). A Lymph node biopsy (proximal to transfer site) as well as a jejunal biopsy (6-12 sites) and a bone marrow aspirate (less or equal to 5 mls, not to exceed a combined volume of 20% of total blood volume and 12 mls/kg/month including the large

bleed for CFSE labeling at day 0) will be obtained at that time (once). An additional 1 ml bleed will be obtained at days 1, 3, 5, 10 post transfer, totaling 4 mls (also following CRPRC blood volume guidelines). The absolute volume bled at day 0 will differ for each animal based on its size and weight, and final volumes be determined once specific animals are assigned to this study.

For the biopsy procedure, animals will be under ketamine and propofol anesthesia. Peripheral blood lymphocytes and lymph node cells will be tested for virus load (co-culture assay, plasma viremia, plasma antigenemia) and anti-viral antibody responses and extensive flow cytometric analysis. Complete blood counts (CBC), including CD4 and CD8 lymphocytes, will be determined at the timepoints listed above on peripheral blood samples to monitor potential hematologic abnormalities that may accompany SIV infection and cellular transfer. When animals are bled, weights will be determined. CRPRC guidelines outlining criteria for euthanasia for retrovirus infected macaques will be followed.

All animals will be necropsied between days 7-14 post adoptive transfer.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require**. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
1	Bleed, adoptive transfer (iv), biopsy (gut/LN/BM), necropsy	4	3
2	SHIV/SIV Infection IN (pathogenic strain), bleed, adoptive Transfer (iv), biopsy (gut/LN/BM), necropsy	4	3
3	SHIV/SIV Infection IN (non-pathogenic strain), bleed, adoptive Transfer (iv), biopsy (gut/LN/BM), necropsy	4	3
4	Bleed, In vitro Infection (SHIV/SIV), adoptive transfer of infected cells (iv), biopsy (gut/LN/BM), necropsy	4	3
5	Challenge with SIV/SHIV virus, bleed, adoptive transfer, biopsy (gut/LN/BM), necropsy	3	3

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

- 1.) Rhesus macaques have proven to be a valuable non-human primate model for investigating many human infectious agents and closely related simian pathogens. Macaques exhibit a fatal AIDS-like disease after exposure to SIV or SHIV. Accordingly, macaques are an important model for analyzing mechanisms of pathogenesis that occur in HIV infection and AIDS. Additionally, the immune system of macaques shares many similarities to that of humans. A non-human primate model will be essential for developing and testing. In humans, investigations of lymphocyte migration during acute and chronic phases of infection are extremely difficult or not possible. Therefore, addressing the issue of stimulation of immune responses and chances of antigen encounter of appropriate T lymphocytes in macaques will contribute to our understanding of anti-viral immune protection.
- 2.) Our previous studies at CRPRC on SIV and SHIV clones and variants indicates that statistically valid information can be obtained from a group of 4 animals inoculated with one virus. However, this project is pilot in nature, and groups of 2 will be inoculated initially, 2 more animals per group will be inoculated thereafter when transferred cells have been detected. As discussed above, we are unable to obtain 4 animals for Group 5, as 3 genotyped animals from an ongoing study will be recruited into our study here. These animals would be necropsied otherwise, and constitute a tremendous resource for us.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building: Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
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Rhesus	Ketamine HCL	10	IM	30 min/day
Rhesus	Isoflorane	1-2%	Inhaled	Duration of procedure (~1 hr)
Rhesus	Telazol	5-8 mg/kg	IM	During Intranasal Inoculation (1)

h) Neuromuscular blocking agents can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

N/A

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

N/A

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

N/A

i) Adverse effects:

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

SIV or SHIV infection of susceptible macaques produces a progressive fatal immunodeficiency disease characterized by hematologic abnormalities, lymphocyte depletion, weight loss and cachexia, an infection with opportunistic pathogens. CRPRC guidelines outlining criteria for euthanasia for virus infection will be followed. Although as published in the literature, no adverse reactions to intracellular dye CFSE have been noted in mice, and no adverse effect on human lymphocytes *in vitro*, there is a potential of adverse effects in rhesus, although unlikely especially given the very short duration of the experiment, and *in vivo* experiments in mice. If animals exhibit adverse effects, the CRPRC staff and veterinarians will treat the animals with any medication to counteract these effects.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

Analgesics, steroids and antibiotics may be given.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? Yes No

(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not

appropriate for your particular study. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search?

10-31-01

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
PubMed	Unlimited	CFSE, Primate, SIV/HIV, migration, in vivo, viability, dye, trafficking
Current Contents	Unlimited	CFSE, Primate, SIV/HIV, migration, in vivo, viability, dye, trafficking

What were your findings with respect to alternative methodologies?

As yet, there are no established methods to study migration of both infected and uninfected T lymphocytes in primates during acute infection or re-exposure, that allows assessment of dissemination of infected and uninfected lymphocytes. These in vivo trafficking studies can not be performed in humans, but only in experimentally infected non-human primates.

Has this study been previously conducted?

Yes No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

k) **Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

Macaques will be euthanized at 7-14 days post autologous adoptive transfer. All lymphoid tissues assessed for migrated populations (CFSE positive) and virus-infected cells after tissue collection.

l) **Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
Rhesus	Overdose	Sodium Pentobarbital	60	IV

m) **Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

Animals will be necropsied at 7-14 days post transfer.

Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

<i>Principal Investigator</i>	<i>Rank / Title</i>	<i>Date</i>
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Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

ANIMAL ROOM SAFETY INFORMATION

Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

PROTOCOL # 9840**EXPIRES:** _____

RUA#: _____

BUA#: Lab _____

CCA#: _____

Identity of Hazard: Investigator Last Name: Department: First Name: Phone: Email: Fax: **Provide a short description of the agent:**

This agent / material is hazardous for: Humans only Animals only Humans and Animals
For which Animal Species?

The agent can be spread by: Blood Feces/urine
 Saliva/nasal droplets Does not leave animal
 Other:

Describe any human health risk associated with this agent:

No human disease related to these viruses has ever been described. However, there is a potential for these viruses to infect humans.

The precautions checked below apply to this experiment:

- The researcher or his/her technicians are responsible for the feeding and care of these animals.
 The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians.
 Cage Stall Water Bottle Animal Carcasses
 Bedding Other:
 Cages must be autoclaved before cleaning.
 Label cages and remove label after decontamination.
 Animal carcasses must be labeled and disposed of as follows:
 Incineration Biohazardous Waste Container
 Bag and Autoclave EH&S will pick-up (2-1493).
 All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:
 Incineration Biohazardous Waste Container
 Bag and Autoclave EH&S will pick-up (2-1493).

Personal Protective Equipment Required:

- The following personal protective equipment must be worn/used in the room:
 Lab Coat/Coveralls Shoe Covers/Booties
 Disposable Gloves Head Cover
 NIOSH Certified Dust Mask Disinfectant footbath
 Eye Protection/Face Shield
 Fitted Respirator Type:
 Other: Describe:
 Personal protective equipment must be removed before leaving the room.
 Personal protective equipment must be discarded or decontaminated at the end of the project
 Hands, arms, and face must be thoroughly washed upon leaving the room
 Full shower, including washing of hair, must be taken upon leaving the room.
 Decontaminate Room (Inform ARS area supervisor when cage and/or room can be returned to general use).

Provide any other information needed to safely work in this room:



