

PROTOCOL FOR ANIMAL USE AND CAREEmail to: campusvet@ucdavis.edu

EH&S USE ONLY

PROTOCOL: 10033
EXPIRES:

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

Species (common names):	Number:	Source:
Rhesus Macaque	48	CNPRC

Project Title	Anti-retroviral therapy in simian AIDS		
Overnight housing location::	CNPRC	Day use:	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator (If investigator maintained, attach husbandry SOP's.)		

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.

This project proposes to determine the effects of PMPA and IL-2 therapy on immune restoration and virus infection in SIV infected rhesus macaques. Procedures include SIV infection, intestinal and lymph node biopsy, intestinal resection, blood collection, bone marrow aspirates, saliva samples, PMPA, IL-2 administration, intestinal, rectal and lung lavages, and jejunal and colonic aspirates.

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.

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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 type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input 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 checked="" 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):	SIV
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	NIH	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	8473

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 pctillman@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.

The main objective of this project is to determine the effect of PMPA (9-[2-(phosphonomethoxy)ethyl] adenine) and the cytokine IL-2 (interleukin 2) therapy on immune restoration and viral infection in lymphoid tissues of acute and chronically SIV (Simian Immunodeficiency Virus) infected rhesus macaques. Most of the studies examining restoration of CD4+ T cells and immune functions are being performed in PBMCs (peripheral blood mononuclear cells) from HIV-1 infected patients. Yet most of the lymphocytes in the body reside in lymphoid tissues. The gut associated lymphoid tissue (GALT) harbors greater than 85% of the lymphoid tissue in the body. Thus evaluation of gut tissues will be critical in determining the true efficacy of anti-retroviral therapy and immune restoration. We propose to examine lymphocytes and immune cells from intestinal lymphoid tissue and compare them with cells in blood and other lymphoid organs. We will examine immunophenotypic changes, cytokine expression, viral variants at the genomic level, gene regulation in lymphoid tissues and viral loads in these cells following anti-retroviral therapy in both chronically and acutely infected animals to determine the efficacy of anti-retroviral therapy at the whole animal level. We will also examine the effect of IL-2 (interlukin-2) administration in conjunction with PMPA therapy on viral loads and immune restoration in SIV infected macaques. Studies of this nature are not feasible in HIV-infected patients following anti-retroviral therapy. The proposed studies will be valuable in determining the immunologic and virologic outcome of PMPA treatment in GALT in comparison with peripheral blood and lymph nodes.

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 checked="" type="checkbox"/> Induced illness, intoxication, or disease
<input type="checkbox"/> LD 50 or ID50 studies.	<input checked="" type="checkbox"/> Survival surgical procedures	<input type="checkbox"/> Death as an endpoint (see i below)
<input checked="" type="checkbox"/> catheters, blood collection, intubation	<input checked="" 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 checked="" type="checkbox"/> endoscopy
<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 total of 48 animals will be used in this study. Forty two animals will be infected intravenously (1000 TCID₅₀), or orally (1 ml virus stock) with either pathogenic SIVmac251 or SIVmac239. Due to the reduced infection rate with oral inoculations, any oral inoculation will be repeated until the animal becomes infected. The PMPA (9-[2(phosphonomethoxy)ethyl]adenine) treatment (30mg/Kg/day, subcutaneously) will be initiated in 12 animals at 1 week post-infection and in 12 animals at 8 weeks post-infection. After 3 months at 30mg/Kg/day PMPA (subcutaneously), animals will have monthly chemistry panels, performed by the CRPRC Clinical Lab, to determine possible PMPA toxicity. If toxicity occurs in an animal receiving PMPA at 30mg/Kg/day, PMPA dosage will drop to 15mg/kg/day (subcutaneously) until time of necropsy. PMPA is a nucleotide analogue with potent anti-retroviral activity. The animals will be monitored for 3 years post infection for the clinical outcome and virologic and immunologic parameters. The endpoint of treatment is 3 years or until development of severe disease. Blood samples at designated time points (preinfection, 1, 4, 8, 24, 36 and 52 weeks and every 4 months post infection) for Clinical Lab evaluation, weekly weight determination and daily CRPRC staff observation of the specially housed, infected animals will be utilized to monitor the animals. PMPA will be given on a daily basis until necropsy. Twelve animals will receive, in addition to daily PMPA (subcutaneously), subcutaneous IL-2 (3 million IU/day) for 1 week after 4 weeks of PMPA therapy. Blood samples (10 to 20 ml) and lymph node and jejunal or colonic biopsies (using endoscopy), will be obtained at various time points (preinfection, 1, 4, 24, 36 and 52 weeks post infection). Biopsies will consist of 12 small (20-25 mg each) tissue **pieces from which 4 to 6 million cells can be isolated. There is the risk of intestinal perforation with jejunal biopsies. When a perforation occurs, a jejunal resection will be performed. These samples will be used to obtain basic information such as T cell subset alterations.**

Jejunal resections will be performed at 2 weeks post infection and after 8 weeks of PMPA therapy. **Animals will undergo full general anesthesia with ketamine at 10mg/kg and isoflurane (at 1-2% inhaled). A ventral midline abdominal incision will be made. The jejunum would be exteriorized and clamped to preserve the vascularity with Doyen forceps. An approximate 3-5 cm section of jejunum would be removed and an anastomosis would be performed using absorbable suture. The abdomen would be lavaged and then closed routinely in 3 layers. Post operatively we would add an analgesic Buprenorphine 0.03mg/kg TID for 3 days. Resections are needed to obtain larger numbers of cells, 30-50 million, in order to perform in vitro assays that require 5-10 million cells for each assay. These samples will be used to obtain more extensive immunological and functional information on the mucosal T cells in the infected animal.**

Six animals will receive PMPA alone and will serve as control animals. All animals will be euthanised as specified in the CRPRC guidelines "criteria for euthanasia of retrovirus infected macaques" when severe disease develops. A complete necropsy will be performed for each animal and peripheral and systemic lymphoid tissues will be prepared for histological, immunohistochemical, flow cytometry, bDNA, PCR and gene analysis.

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	After 1 week of SIVmac251 infection, daily PMPA administered subcutaneously, jejunal and lymph node biopsy, jejunal resection, blood collection, bone marrow aspirates (1 ml, requested as needed), rectal lavages and saliva collection (1 ml ea).	6	3
2	After 8 weeks of SIVmac251 infection, continuous 30mg/Kg/day PMPA administered subcutaneously, jejunal and lymph node biopsy, jejunal resection, blood collection, bone marrow aspirates (1 ml, requested as needed), rectal lavages and saliva collection.	6	3
3	6 animals will receive 30mg/Kg/day PMPA only, administered subcutaneously, and no viral infection. These are control animals and will receive PMPA daily. Perform jejunal and lymph node biopsy, jejunal resection, blood collection, bone marrow aspirates (1 ml, requested as needed), rectal lavages and saliva collection.	6	3

4	After 8 weeks SIVmac239 infection, daily 30mg/Kg/day PMPA administered subcutaneously, jejunal and lymph node biopsy, jejunal resection, blood collection, bone marrow aspirates(1 ml, requested as needed), rectal lavages and saliva collection.	6	3
5	6 animals with SIVmac239 infection only for 52 weeks, jejunal and lymph node biopsy, jejunal resection, blood collection, bone marrow aspirates(1 ml, requested as needed), rectal lavages and saliva collection. Necropsy at 52 weeks post infection.	6	3
6	6 animals with SIVmac251 infection, 30mg/Kg/day PMPA, administered subcutaneously, after 1 week of infection and one week concurrent IL-2 administration (subcutaneous administration, 3 million IU/day) (after 4 weeks PMPA) subcutaneously. Jejunal and lymph node biopsy, jejunal resection, blood collection, bone marrow aspirates(1 ml, requested as needed), rectal lavages and saliva collection.	6	3
7	6 animals with SIVmac251 infection, continuous PMPA (daily subcutaneous administration at 30mg/Kg/day) after 8 weeks of infection and one week concurrent IL-2 administration at 3 million IU/day (after 4 weeks PMPA) subcutaneously. Jejunal and lymph node biopsy, jejunal resection, blood collection, bone marrow aspirates(1 ml, requested as needed), rectal lavages and saliva collection.	6	3
8	6 animals with SIVmac251 infection, only. Jejunal and lymph node biopsy, blood collection, jejunal resection, bone marrow aspirates(1 ml, requested as needed), rectal lavages and saliva collection.	6	3

Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p>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.</p>
2	<p>Minor stress or pain of short duration</p> <p>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</p>
3	<p>Moderate to severe distress</p> <p>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</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p>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.</p>

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?

Intestinal abnormalities including nutrient malabsorption, diarrhea and wasting are common features of HIV-1 infection. Studies on the effect of anti-retroviral therapy on HIV associated enteropathy are limited due to difficulties in obtaining sufficient amounts of intestinal tissues for analysis at different time points following viral infection and therapy. SIV infected rhesus macaques are extremely valuable as a suitable animal model to examine the effect of PMPA and IL-2 therapy on the restoration of the immune compartment and function in

gut associated lymphoid tissues of SIV infected rhesus macaques. Our preliminary studies indicate that immunophenotypic and functional alterations occurring in intestinal lymphocytes following SIV infection do not parallel those seen in the peripheral blood. Thus the effects of antiretroviral therapy on T cell dynamics in the gastrointestinal lymphoid tissue and lymphoid tissues at other sites independent of peripheral blood warrants examination in order to determine the true efficacy of PMPA.

IL-2 treatment is known to stimulate viral replication and activate T cells. We propose to ascertain if PMPA treatment and concurrent IL-2 administration will eliminate a greater number of infected cells in gut lymphoid tissue and alter immune restoration and function as compared to PMPA treatment alone.

SIV infected macaques will be the most relevant animal model to study the pathogenesis of HIV-1 associated enteropathy. There is no comparable lentivirus infection animal model available that is suitable for the studies of pathologic and functional alterations in intestinal epithelial and lymphoid populations during the entire course of disease development.

Forty eight animals will be used for this study. Of these, forty two will be infected with either pathogenic SIVmac239 or SIVmac251 covering the acute (1 week) and chronic (8 week) infection and treated daily with PMPA until necropsy. Twelve of these animals will also receive IL-2 treatment, after 4 weeks of PMPA treatment, for 1 week. Six animals will be used as controls with six being infected with SIVmac239, six being infected with SIVmac251 and six receiving PMPA only. Animals will be euthanized at the end of each treatment period. Four animals will be used for each animal group as this is the minimum number required to get reliable and useful information.

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

Building:

CNPRC Surgery

Room:

Surgical suite

Who will be the surgeon?

CNPRC Veterinary staff

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?
M. mulatta	telazol	5 mg/kg	IM	before biopsy procedure
M. mulatta	buprenorphine	0.01-0.03 mg/Kg	IM	BID for 3 days
M. mulatta	Midazolam	.05-.1 mg/kg	IM	before biopsy procedure
M. mulatta	isoflurane	1-2%	inhaled	During biopsy procedure
M. mulatta	Ketamine	10/mg/Kg	IM	before biopsy procedure

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?

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

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

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)

Discomfort may accompany bone marrow aspirates, intestinal biopsies.

Blood collection may be associated with minimal discomfort.

Perforation can occur with intestinal biopsies. If a perforation of the jejunum occurs a resection will be performed to ameliorate the condition of the animal.

Possible adverse effects of jejunal resection include risk of infection or peritonitis. Antibiotics will be used as needed by veterinary staff to ameliorate ant resulting infection.

Animals will be euthanized according to CRPRC criteria for euthanasia of SIV infected macaques. This would include weight loss of >15% in 2 weeks, persistent leukopenia, total WBC<3,000, opportunistic infections that do not respond to therapy, dehydration >7% and not responsive to oral hydration therapy for 3 days, lymphopenia, abdominal lesions and severe depression (obtusion).

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.

Yes, analgesics or any post-operative procedures may be utilized as deemed necessary by the attending veterinarian .

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 [x] 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:

*Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that this use of animals is not **unnecessarily** duplicative of other studies.*

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit

http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu)

or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwood@ucdavis.edu)

What was the date on which you conducted this search?

June 2002

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
Pub Med	1991-present	AIDs, HIV, SIV, Intestine
Medline	1991-present	AIDS, SIV, Intestine, jejunum
Current Contents	1991-present	AIDS, SIV infection, intestine

What were your findings with respect to alternative methodologies?

There are no known alternatives to the procedures used in this study	
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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.

At the end of each treatment period and animals with SAIDS will be euthanised.
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k) **Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

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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 macacques	deep ketamine anesthesia followed by barbiturate overdose	Sodium pentobarbital	60 mg/kg	I.V.

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

Use for other approved projects

ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL # 10033
EXPIRES: _____

RUA#: _____

 BUA#: 0400

CCA#: _____

 Identity of Hazard:

 Investigator Last Name: Department:

 First Name: Phone:

 Email: Fax:
Provide a short description of the agent:

SIV (simian immunodeficiency virus) is a blood born lentivirus that cause fatal immunodeficiency (AIDS) in rhesus macaques. . It is genetically similar to HIV. SIV can infect humans but it is unknown whether it can cause disease..

 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: mucosal contact (eye/ mouth/nose/ genital)

Describe any human health risk associated with this agent:

SIV can infect humans; thus, it could possibly cause fatal AIDS-like disease in humans. SIV-infected humans have generated infectious virus and antibodies to SIV. There have been no reports of disease seen in SIV-infected 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: disposable gown/coveralls
- 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:

Biosafety level 2+ (BSL2+) precautions must be followed at all times