PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
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<td>Last Name:</td>
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<td>Department:</td>
<td>Department:</td>
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<td>Phone / Fax:</td>
<td>After hrs. #:</td>
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<table>
<thead>
<tr>
<th>Species (common names):</th>
<th>Number:</th>
<th>Source:</th>
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<tbody>
<tr>
<td>Rhesus Macaque</td>
<td>24</td>
<td>CRPRC</td>
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**Project Title**: Pathogenesis of Simian AIDS

**Overnight housing location**: CRPRC  **Day use only**: CRPRC

Animals will be maintained by: [x] Vivarium [ ] 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.

The main objective of this project is to determine the effects of anti-retroviral (PMPA) therapy on immune restoration in lymphoid tissues of SIV infected rhesus macaques. Procedures will include SIV infection, endoscopy for intestinal biopsy, blood sample collection, breath hydrogen collection for analysis of sucrose absorption, bone marrow aspirates and lymph node biopsy.

**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.

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

<table>
<thead>
<tr>
<th>Sick Animals</th>
<th>Dead Animals</th>
<th>Pest Control</th>
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<tbody>
<tr>
<td>[x] Call Investigator</td>
<td>[x] Call Investigator</td>
<td>[ ] Call Investigator</td>
</tr>
<tr>
<td>[ ] Clinician to treat</td>
<td>[x] Save for Investigator</td>
<td>[ ] OK to use pesticides</td>
</tr>
<tr>
<td>[ ] Terminate</td>
<td>[ ] Bag for disposal</td>
<td>[ ] No Pesticides in animal area</td>
</tr>
<tr>
<td>[x] Necropsy</td>
<td>[x] Necropsy</td>
<td></td>
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</table>

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

- Infectious Agents? [x] Yes [ ] No  Agent(s): SIV
- Radioisotopes? [ ] Yes [x] No  Agent(s): 
- Chemical Carcinogens? [ ] Yes [x] No  Agent(s): 
- Toxic Chemicals? [ ] Yes [x] No  Agent(s): 

University of California, Davis
Printed 7/21/2004 11:00:12 AM Page 1
The main objective of this project is to determine the effect of PMPA therapy on immune restoration in lymphoid tissues of SIV infected rhesus macaques. Most of the studies examining repletion of CD4+ T cells and restoration of immune functions are being performed in the peripheral blood from HIV-1 infected patients. However, most of the lymphocytes reside in lymphoid tissues. The gut associated lymphoid tissue harbors >85% of the lymphoid tissue in the body. Thus evaluation of these tissues will be critical in determining the efficacy of anti-retroviral and immune restoration. We propose to examine lymphocytes and immune cells from intestinal lymphoid tissue and compare with cells in blood and other lymphoid organs. We will examine immunophenotypic changes, cytokine expression, viral variants and anti-viral activity in these cells following anti-retroviral therapy and determine the efficacy of anti-retroviral therapy at the whole animal level. Studies of this nature are not feasible in HIV-infected patients following antiretroviral therapy.
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.)*

Twenty-four animals will be infected by different routes (intravenous, intravaginally, orally) with 1 ml of 1000 TCID50 doses pathogenic SIV for 1, 2, and 12 weeks before being treated with PMPA (30mg/Kg/day in 1ml subcutaneously) for different lengths of time (4 weeks to 24 weeks). The animals will be monitored for the development of clinical disease (rapid weight loss, listlessness, diarrhea, high viral load, dehydration and being non-responsive to therapy for opportunistic infections). Blood samples (5 to 10 ml) and lymph node and jejunal biopsies (using endoscopy) will be obtained at various time points (pre-infection, 1, 4, 8, 12, 16, 20 weeks post infection). Animals will be euthanised at two years post infection as specified in the CRPRC guidelines "criteria for euthanasia of retrovirus infected macaques". A complete necropsy will be performed for each animal and peripheral and systemic lymphoid tissues will be prepared for histological, immunohistochemical, flow cytometry, PCR or molecular 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 week SIV infection, 4 weeks PMPA administered subcutaneously, jejunal and lymph node biopsy, blood collection, bone marrow aspirates at 1, 4, 8, 12, 16, 20 weeks post infection</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>2 weeks SIV infection, 4 weeks PMPA administered subcutaneously jejunal and lymph node biopsy, blood collection, bone marrow aspirates at 1, 4, 8, 12, 16, 20 weeks post infection</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>12 weeks SIV, 4 weeks PMPA administered subcutaneously jejunal and lymph node biopsy, blood collection, bone marrow aspirates at 1, 4, 8, 12, 16, 20 weeks post infection</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1 week SIV infection, 24 weeks PMPA administered subcutaneously jejunal and lymph node biopsy, blood collection, bone marrow aspirates at 1, 4, 8, 12, 16, 20 weeks post infection</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>2 weeks SIV infection, 24 week PMPA administered subcutaneously jejunal and lymph node biopsy, blood collection, bone marrow aspirates at 1, 4, 8, 12, 16, 20 weeks post infection</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>12 weeks SIV infection, 24 week PMPA administered subcutaneously jejunal and lymph node biopsy, blood collection, bone marrow aspirates at 1, 4, 8, 12, 16, 20 weeks post infection</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>
Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| 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?

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 is 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 will be extremely valuable in examining the effect of PMPA therapy on the restoration of immune restoration and function in gut associated lymphoid tissues of SIV infected rhesus macaques. Our results indicate that immunophenotypic and functional alterations occurring in intestinal lymphocytes following SIV infection are not adequately mirrored in the peripheral blood. Thus the effects of anti-viral treatments warrant an examination of T cell dynamics in the gastrointestinal lymphoid tissue and lymphoid tissues at other sites independent of peripheral blood in order to determine the efficacy of anti-retroviral treatments.

Twenty four animals will be infected with pathogenic or nonpathogenic SIV covering the acute (1 and 2 weeks) and chronic infection (12 weeks) and treated for different periods ( 4 to 24 weeks) with PMPA. Animals will be euthanized at the end of each treatment period. Biopsies and blood samples will be collected at various time points before, after infection, and after treatment. Lymphoid cells will be isolated from intestinal and other lymphoid tissues for immunohistochemical and functional analysis by flow cytometry. Four animals will be used for each group and this is the minimum number required to get reliable and useful information.

Our previous studies have shown that SIV infected rhesus macaques represent the best animal model for the studies on HIV associated enteropathy. The histopathologic and functional alterations in intestinal mucosa and clinical disease in SIV infected macaques are strikingly similar to those observed in HIV-1 infection. Small intestinal morphology, epithelial and lymphoid cell subset distributions in macaques are very comparable to those observed in human intestinal tissues. These findings demonstrate that with 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.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. mulatta</td>
<td>telazol</td>
<td>5 mg/kg</td>
<td>IM</td>
<td>once/day</td>
</tr>
</tbody>
</table>

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 and intestinal biopsies, however animals are anesthetized during the entire procedure.

Discomfort may accompany SIV infection when the infection reaches its clinical course. There may be dehydration, opportunistic infections, diarrhea, and hypothermia. All these symptoms will be treated accordingly by attending veterinarian.

Blood collection may be associated with minimal discomfort.

Animals will be euthanized according to CRPRC criteria for euthanasia of SIV infected macaques.

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 may be given 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:

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?  

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

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
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<tbody>
<tr>
<td>Medline</td>
<td>ten year</td>
<td>AIDS, SIV, Intestine, jejunum</td>
</tr>
<tr>
<td>Melvyl</td>
<td>ten year</td>
<td>AIDS, SIV infection, intestine, jejunum</td>
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</tbody>
</table>

What were your findings with respect to alternative methodologies?

Has this study been previously conducted?  

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?

At the end of each treatment period and animals with SAIDS will be euthanized

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhesus macacques</td>
<td>deep ketamine anesthesia followed by barbiturate overdose</td>
<td>Sodium pentobarbital</td>
<td>60 mg/kg</td>
<td>I.V.</td>
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</tbody>
</table>

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

Use for other approved projects
Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don’t need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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Occupational Health Program:

Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://clueless.ucdavis.edu/health/](http://clueless.ucdavis.edu/health/) or read the UC Davis Policy & Procedure Manual 290-25.

Training:

Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at [http://clueless.ucdavis.edu/](http://clueless.ucdavis.edu/).
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.

Principal Investigator | Rank / Title | Date
--- | --- | ---

** Conditions necessary for Committee Approval:

Final Disposition of this protocol:

________ Approved

________ Not Approved

________ 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.

Campus Veterinarian

Date
ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL # 9485
EXPIRES: ________

Identity of Hazard: SIV

Investigator Last Name: ___________________________  Department: ___________________________
First Name: ___________________________  Phone: ___________________________
Email: ___________________________  Fax: ___________________________

Provide a short description of the agent:
SIV is a retrovirus that is 90% similar to HIV, the viral agent that causes AIDS.

This agent / material is hazardous for: [ ] Humans only  [ ] Animals only  [ x ] Humans and Animals

The agent can be spread by:
[ x ] Blood  [ ] Feces/urine  [ ] Does not leave animal
[ x ] Saliva/nasal droplets  [ ] Mucosal contact (eye/mouth/nose/genitalia
[ ] Other:

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:

[ x ] Cages must be autoclaved before cleaning.
[ ] Label cages and remove label after decontamination.
[ x ] Animal carcasses must be labeled and disposed of as follows:
   [ ] Incineration  [ xx ] Biohazardous Waste Container
   [ ] Bag and Autoclave  [ ] EH&S will pick-up (2-1493).

[ x ] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:
   [ ] Incineration  [ x ] 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  [ x ] Shoe Covers/Booties
   [ x ] Disposable Gloves  [ x ] Head Cover
   [ ] NIOSH Certified Dust Mask  [ ] Disinfectant footbath
   [ x ] Eye Protection/Face Shield  [ ]
   [ ] Fitted Respirator  Type: ___________________________
   [ x ] Other: ___________________________  Describe: disposable gown/coveralls

[ x ] Personal protective equipment must be removed before leaving the room.
[ ] Personal protective equipment must be discarded or decontaminated at the end of the project
[ x ] 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