PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CNPRC

Investigator

Last Name: ___________________________  Last Name: ___________________________

First: ___________________________  First: ___________________________

Middle: ___________________________  Middle: ___________________________

email: ___________________________  email: ___________________________

Department: ___________________________  Department: ___________________________

Phone: ___________________________  Phone: ___________________________

Fax: ___________________________  Fax: ___________________________

Contact

PROTOCOL # ___________________________

EXPIRES: ____________

Species (common names):  Number:  Source:

Rhesus monkey  48  Primate Center

(dams+fetuses)

Project Title  Pathogenic Potential of Recombinant CMV in Fetal Monkeys

Overnight housing location:  Primate Center  Day use only:  

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.

Novel CMV-based vaccines will be transferred into fetal monkeys in the late first trimester and blood and amniotic fluid samples collected during gestation. Maternal blood samples will also be collected during gestation. Hysterotomies will be performed at ~140 days gestation.

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.

None

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

Sick Animals  Dead Animals  Pest Control

[x] Call Investigator  [x] Call Investigator  [x] Call Investigator

[ ] Clinician to treat  [ ] Save for Investigator  [ ] OK to use pesticides

[ ] Terminate  [ ] Bag for disposal  [x] No Pesticides in animal area

[ ] Necropsy  [ ] Necropsy

Hazardous Materials (only if in the animal room):

Infectious Agents?  [x] Yes  [ ] No  Agent(s): Rhesus CMV (BAUA 0547)

Radioisotopes?  [ ] Yes  [x] No  Agent(s): 

Chemical Carcinogens?  [ ] Yes  [x] No  Agent(s): 

Toxic Chemicals?  [ ] Yes  [x] No  Agent(s): 

Is the project already funded?  [ ] Yes  [X] No

Previously approved?  [X] Yes  [ ] No

Proposed Funding Source: NIH

Previous protocol number (if any): 8835

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

[ ] Lab Animal Health Clinic (2-0514)
[ ] VMTH Large Animal Field Service (2-0292)
[ ] California Primate Research Center (2-0447)
[ ] Another Veterinarian

If you checked “Another Veterinarian”, please provide:

Veterinarian: __________________________ Address: __________________________

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.

There is a clear and pressing need for HIV vaccines, and bold new concepts in vaccine design are essential to halt the onset of the pandemic. Cytomegalovirus (CMV) is a member of the herpesvirus family that offers potential as a novel vaccine vector. Human CMV has low pathogenic potential in the immunocompetent host, and establishes a persistent infection for the lifetime of the host. Thus, CMV can be exploited in order to generate and sustain immune responses to heterologous antigens such as HIV or SIV. In particular, expression of HIV or SIV proteins in the context of the CMV genome may elicit robust anti-HIV immune responses at both local and systemic levels. The safety of this concept will be tested in our established rhesus monkey model of rhesus CMV infection.

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

- [ ] Monoclonal Antibody Production
- [ ] Polyclonal Antibody Production
- [ ] LD 50 or ID50 studies.
- [ ] catheters, blood collection, intubation
- [ ] Prolonged restraint (8 hrs+)
- [ ] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [ ] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] Behavioral modification.
- [ ] Special diets; food or water treatment.
- [ ] Induced illness, intoxication, or disease
- [ ] Death as an endpoint (see h below)
- [ ] Trapping, banding or marking wild animals
- [ ] Aversive conditioning.

** 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.)

Gravid adults will be screened for endogenous viruses (~2 ml blood sample collected from a femoral vessel), then selected for study (N=24). In Years 1 and 2, 12 animals will be used, 8 with fetal inoculation (2 vaccines tested, N=4 per vaccine) and 4 will be controls (no inoculations). In Year 3, 12 animals will be used, N=4 per vaccine. Each novel CMV vaccine expressing SIV antigens will be injected into fetal monkeys at ~50 days gestation (fetal intraperitoneal administration via ultrasound guidance; 0.3 ml), using established techniques. All fetuses will be sonographically monitored approximately every 5-10 days post-inoculation until hysterotomy at ~140 days gestation. Fetal samples (~0.5-1 ml blood, 1-2 ml amniotic fluid) will be collected every 10 days beginning at 70 days gestation using routine ultrasound-guided techniques. Samples will also be collected from the dams (~3-12 ml; femoral vessel, volumes will not exceed acceptable limits, based on body weight) prior to fetal inoculation, then every 10 days until hysterotomy (plasma, serum; CBCs pre-inoculation, day 100 and at delivery). The dams will be administered ketamine hydrochloride (10 mg/kg) for these evaluations. At ~140 days gestation, fetal tissues will be removed by hysterotomy and a complete fetal tissue harvest performed using established techniques. The dams with inoculated fetuses will either be re-bred for future pregnancies using established Primate Center SOPs or be euthanized for a complete necropsy at the end of the study.

d: 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>Controls -- fetal blood and amniotic fluid collection, maternal blood samples, hysterotomy</td>
<td>4 dams + 4 fetuses = 8</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Fetal inoculation, fetal blood and amniotic fluid collection, maternal blood samples, hysterotomy</td>
<td>20 dams + 20 fetuses = 40</td>
<td>3</td>
</tr>
</tbody>
</table>

### Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Little or no discomfort or stress</td>
</tr>
<tr>
<td></td>
<td><em>Examples:</em> 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.</td>
</tr>
<tr>
<td>2</td>
<td>Minor stress or pain of short duration</td>
</tr>
<tr>
<td></td>
<td><em>Examples:</em> 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</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe distress</td>
</tr>
<tr>
<td></td>
<td><em>Examples:</em> 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</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain near, at or above the pain tolerance threshold</td>
</tr>
<tr>
<td></td>
<td><em>Examples:</em> 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.</td>
</tr>
</tbody>
</table>

Further descriptions of these categories are included in the instructions following this document.
e) Rationale for species and numbers: How did you determine that the species choice was appropriate and the number of animals in the groups above was the minimum number necessary to achieve sound scientific results?

Monkeys are the only appropriate model for these studies because of reproductive, developmental, and immune system similarities when compared to humans. Based on our experience with this model, the number chosen is the minimum required in order to adequately assess group differences. The intent of this study is to test whether recombinant CMV engineered to express SIV proteins retain pathogenic potential following fetal inoculation. The focus will be on testing different variants of rhesus CMV for inducing CMV disease in fetuses compared to wild-type virus. It is important to note that we are not creating an infectious SIV, rather just expressing SIV proteins. The expression of these proteins should not alter the natural history of rhCMV.

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

<table>
<thead>
<tr>
<th>Building</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRPRC animal quarters</td>
<td>Surgery suite</td>
</tr>
</tbody>
</table>

Who will be the surgeon?

CRPRC veterinarians

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>Rhesus</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>dams: ~15x</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>5-8</td>
<td>IM</td>
<td>5-6 times</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Isoflurane</td>
<td>to effect</td>
<td>inhal.</td>
<td>Cesarean-section, 1x</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Buprenorphine</td>
<td>0.01-0.03</td>
<td>IM</td>
<td>Post-surgery for dams</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Oxymorphone</td>
<td>0.15</td>
<td>IM</td>
<td>Post-surgery for dams</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)

We do not anticipate any adverse effects based on our extensive experience with this model. Multiple genetic variants of rhesus CMV (RhCMV) are ubiquitous in the rhesus breeding colony, and there are no clinical symptoms associated with RhCMV infection in immunocompetent animals. We have previously documented the fetal neuropathogenesis that can be induced by rhCMV, and will be monitoring sonographically for this outcome, should it occur. By harvesting the fetuses at 140 days gestation, we will avoid any potential adverse effects that could result in term offspring.
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.

See comments above. There are no other adverse effects anticipated or procedures planned that would require administration of analgesics or anesthetics other than those described above.

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?  

9/20/02

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>
</tr>
</thead>
<tbody>
<tr>
<td>PubMed</td>
<td>1980 to current</td>
<td>Fetus, congenital disease, animal models, CMV, SIV, vaccine</td>
</tr>
<tr>
<td>Reference Update®</td>
<td>Most recent publications</td>
<td>Fetus, congenital disease, animal models, CMV, SIV, vaccine</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There are none that would allow us to investigate the questions we propose to address. A primate model is essential in order to determine the potential for pathogenicity of the vaccines under evaluation. This model is ideal for these studies, and is the most rigorous test of safety for potential human application.

Has this study been previously conducted?  

[ ] Yes  [x] No

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

The studies outlined are novel and have never been conducted in the manner proposed.

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

The dams with inoculated fetuses will be euthanized at the end of the study. Those with control fetuses will be returned to the breeding colony two weeks post-hysterotomy.

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>
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</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Overdose</td>
<td>Pentobarbital</td>
<td>60</td>
<td>IV</td>
</tr>
</tbody>
</table>

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

See comments above.
n) **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.

----------------------------------  -----------------------------------  
Professor                                             Rank / Title               Date

----------------------------------  
CRPRC Director                           Date

Committee Use Only Below

** 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.

Identity of Hazard: Rhesus cytomegalovirus (RhCMV)

Investigator Last Name: ___________________________  First Name: ___________________________

Department: ___________________________  Phone: ___________________________

Email: ___________________________  Fax: ___________________________

Provide a short description of the agent:

RhCMV has been isolated from rhesus macaques at the Primate Center; this isolate is serologically and genetically related to human CMV.

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

For which Animal Species? Monkeys

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

Describe any human health risk associated with this agent:

For RhCMV, the potential for infection and disease in humans is not known. Human CMV presents little risk to immunocompetent individuals when handled in a normal laboratory setting. Human CMV can present a risk to immunodeficient individuals and seronegative women who are pregnant.

The precautions checked below apply to this experiment:

**Standard CRPRC conditions for handling and housing applies.**

[ ] 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.