

PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CNPRC

EH&S USE ONLY
PROTOCOL #_10331_
EXPIRES: _____

Investigator	
Last Name:	
First:	
Middle:	
email:	
Dept.:	
Phone:	
Fax:	

Contact	
Last Name:	
First:	
Middle:	
email:	
Dept.:	
Phone:	
Fax:	

Species (common names):	Number:	Source:
Rhesus macaque	56 (5 years)	CNPRC

Project Title	Cytomegalovirus as a Vaccine Vector for SIV Immunization		
Overnight housing location::	CNPRC	Day use only :	
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 that are seropositive for rhesus cytomegalovirus (RhCMV) will be infected with a pathogenic isolate of SIV (SIVmac 251). For Years 1 and 2, animals will be inoculated intra-rectally (IR) with serial dilutions of SIV to determine the minimal animal infectious dose. For Years 3-5, animals will be inoculated IV with a recombinant variant of RhCMV containing defined deletions within the genome. 2 Animals will then be inoculated (IR) 2 weeks later with the minimal animal infectious dose of SIV. The goal is to determine the pathogenic potential of the recombinant RhCMV.

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.

Animals will require infectious 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 checked="" 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):	SIV, RhCMV
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):	

Is the project already funded? Yes No
 Proposed Funding Source: NIH

Previously approved? Yes No
 Previous protocol number:

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

Lab Animal Health Clinic (2-0514) California National Research Center (2-0447)
 VMTH Large Animal Field Service (2-0292) 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.

Intent: 40 million people are infected with HIV worldwide. That number will only continue to escalate unless intervention methods are devised that can interrupt the rapid spread of the virus. There is clear and pressing demand for effective HIV vaccines that can protect from infection and/or disease. Vaccine efforts to date have been limited because there is no clear indication that the strategies will work. Vaccine studies in non-human primates offer some hopeful signs, but unequivocal success remains elusive. Bold, new paradigms are required to stem the HIV pandemic. The basis for this proposal is that the patterns of infection of one virus, cytomegalovirus (CMV), can be exploited to generate vigorous immune responses against HIV. CMV is a member of the herpesvirus family. Like all herpesviruses, CMV establishes a persistent infection for the life of the host. Clinical outcomes are rare in those with a functional immune system. However, CMV can be a serious cause of morbidity and mortality in those that lack immune competence, including those coinfecting with HIV, immunosuppressed transplant recipients, and congenitally infected fetuses. The combination of normally low pathogenicity and persistence imply that expression of foreign genes in the backbone of the CMV genome will elicit robust immune responses against the foreign antigens. The non-human primate model of CMV offers the possibility of testing this concept in a relevant primate host. An important consideration for applicability of this vaccine strategy to humans is to minimize any risk that the engineered variant of CMV would have in those without a functional immune system. The intent of this project is to demonstrate that engineered variants of rhesus cytomegalovirus (RhCMV) expressing lack the full pathogenic potential of wild-type RhCMV.

Hypothesis: The hypothesis is presented that expression of SIV genes within the context of the RhCMV genome will elicit protective anti-SIV immune responses. Further, immune responses will be augmented when RhCMV variants lacking critical immunomodulatory functions are used to express SIV antigens. According to this hypothesis, a balance can be achieved whereby the capacity of RhCMV for persistence can be exploited to elicit protective immune responses to SIV challenge. The ability to maintain anti-SIV will be balanced by an attenuation of RhCMV's reactivation and disease potential. This proposal will test whether deletion of specific RhCMV genes can attenuate viral virulence.

The macaque model presents a well-developed system to test whether human CMV (HCMV) could be used as a vaccine vector. Infection of rhesus macaques with RhCMV is a strong recapitulation of HCMV in terms of natural history and virus-host interactions. The course of RhCMV infection closely parallels that of HCMV in both immunocompetent and immunodeficient hosts. The RhCMV genome has been fully sequenced, is essentially co-linear with HCMV, and is amenable to genetic manipulation. Expression cassettes have been engineered into the RhCMV genomic backbone and are expressed in vitro and in vivo. The constructs are genetically stable and elicit immune responses.

Objectives: (1) Determination of minimal animal infectious dose of SIVmac 251 following intra-rectal inoculation. (2) Characterize the pathogenic potential of deletions variants of RhCMV that will represent the RhCMV vector backbone for future immunization studies. The variants of RhCMV will contain deletions of one or more RhCMV genes implicated in modulation of host immune responses.

Significance: The impetus to develop RhCMV (a model for HCMV) as an expression vector for heterologous antigens is that its complex life cycle might be effectively exploited to create novel vaccine strategies. The combination of low pathogenicity and persistent gene expression at multiple sites implies that de novo immune responses can be generated and sustained at both systemic and local levels. Recent data that HCMV can reinfect healthy seropositive hosts demonstrate that prior seroimmunity does not preclude widespread applicability of HCMV as a vaccine vector. Accordingly, infection with a recombinant HCMV may elicit broad immunity against HIV that could protect against multiple routes of infection, including mucosal surfaces. Infection of rhesus macaques with RhCMV is an excellent model for HCMV persistence and pathogenesis. If successful, this proposal will lead to novel vaccine strategies against HIV.

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 checked="" type="checkbox"/> LD 50 or ID50 studies. | <input type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see h 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.)

Years 1-2) Juvenile/adult macaques (>1 year, male) (n=20 total) will be used for these studies. Blood samples (10 ml collected from a femoral vessel from anesthetized (ketamine) animals) will be collected from animals once per month for 2 months prior to SIV inoculation and on the day of inoculation. Blood will be processed for plasma and PBMC. Animals will be divided into 5 groups (I-V) of 4 monkey/group. Each group will be inoculated intra-rectally with SIVmac 251. Fasted animals will be anesthetized and placed on their stomachs with the pelvic region slightly elevated. A feeding tube (2.7mm x 41 cm; Sherwood Medical, St. Louis, MO) will be gently inserted into the rectum a distance of 15 - 20 cm. After insertion of the tube, a syringe containing SIV will be attached to the tube and the inoculum will be slowly injected into the rectum. After delivery of the inoculum, the tube will be flushed with 3 ml of RPMI-1640 media (without fetal bovine serum) and then slowly withdrawn. Animals will be left in place with the pelvis slightly elevated for 10 minutes.

For Groups I - III, the inoculum will consist of serial dilutions of SIV (1000, 100, and 10 50% tissue culture infectious doses -TCID₅₀ for Groups I - III, respectively) in 2 ml of RPMI-1640 media plus 10% fetal bovine serum. Groups I - III will be inoculated in Year 1. Groups IV and V will be inoculated in Year 2. The titers of infectious virus for Groups IV and V will be dependent on the results obtained with Groups I - III during Year 1. The goal is to identify the minimum titer of SIV required to establish infection in all four animals of each group. Establishment of infection will be determined by a significant drop in CD4⁺ T-cell numbers and development of persistently high SIV loads in plasma (>10⁶ copies/ml of plasma). Groups IV and V monkeys will be used to refine the minimum animal infectious dose according to the results obtained in Year 1.

Blood draws will be obtained every week for the first eight weeks, then every other week thereafter. Blood draws will not exceed 12ml/month/kg body weight. Blood will be processed for plasma and PBMC (dependent on volume of blood). All animals will be necropsied at 6 months, unless signs of simian AIDS warrant earlier euthanasia. The time of euthanasia will be determined in consultation with the veterinary staff at the CNPRC.

Years 3-5) Juvenile/adult macaques (0.5 - 1 year, male) (n=36 total, 12/year) that are seronegative for RhCMV infection will be used for these studies. Blood samples (10 ml collected from a femoral vessel from anesthetized (ketamine) animals) will be collected from animals once per month for 2 months prior to RhCMV inoculation and on the day of inoculation. Blood will be processed for plasma and PBMC (dependent on volume of blood). Animals will be divided into 3 groups of animals per year (4 animals/group). Animals will be inoculated (IV) with recombinant RhCMV variants (10⁶ plaque forming units in 0.3 ml of DMEM media without fetal bovine serum). The variants will be engineered such that specific gene(s) of RhCMV have been deleted. Viral genes targeted for deletion include those postulated to disrupt host cell activation, signaling, and trafficking. The deletions should reduce the pathogenic potential of RhCMV, compared to wild-type RhCMV. Three variants will be evaluated per year (4 monkeys/group) during Years 3 - 5. These variants will not express any heterologous antigens. As such, they will represent the viral backbone for subsequent immunization studies beyond the scope of this proposal. The goal of the studies in Years 3 - 5 is to demonstrate

that the deletion variants have reduced capacity to induce disease in immunodeficient macaques, similar to the goal of the fetal inoculations (Years 3 - 5).

Two weeks post RhCMV inoculation, animals will be inoculated with SIVmac 251 by the intra-rectal route (described above). The titer of SIV will be based on the pattern of SIV infection observed during the titration experiments (Years 1-2, described above).

Following RhCMV inoculation, blood draws will be obtained once per week for six weeks, and then every other week until necropsy at 6 months, unless signs of simian AIDS warrant earlier euthanasia. Blood will be processed for plasma and PBMC (dependent on volume of blood). Blood draws will not exceed 12ml/month/kg body weight. Bronchioalveolar lavages (BAL) and genital/saliva swabs will be collected on the day of RhCMV inoculation and at each time of blood collection post RhCMV inoculation. Oral swabs will be obtained by running a Dacron swab (Fisher, Pittsburgh, PA) inside the lower lip, into the buccal pouch, and along the gumline. Genital swabs will be obtained by inserting the swab into the prepuce of male animals. BAL will be performed by CNPRC veterinary staff according to Standard Operating Procedure (SOP) II-29. Phosphate buffered saline will be used for the lavage. The lavage volume will be based upon the size of the animal (0.5 - 3 ml/kg body weight). BAL and swabs will be processed for viral DNA. All animals will be necropsied at 6 months, unless signs of simian AIDS warrant earlier euthanasia. The time of euthanasia will be determined in consultation with the veterinary staff at the CNPRC.

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
I - V: SIV titration	SIV inoculation (intra-rectal), venous blood draws/ketamine	20 total (12 in Year 1, 8 in Year 2)	3
Pathogenes is of variant RhCMV	RhCMV inoculation (intravenous), SIV inoculation (intra-rectal), venous blood draws, oral/genital swab, Bronchioalveolar lavage/ketamine	36 total (12 each in Years 3-5)	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 the species choice was appropriate and the number of animals in the groups above was the minimum number necessary to achieve sound scientific results?

HIV disease has reached the pandemic stage with no predicted change in the spread of the virus, particularly in Africa and Asia. A vaccine represents the most cost-effective way to slow the spread of the virus, if not preventing infection and/or disease. No such vaccines currently exist, and new paradigms are required. The non-human primate models of SIV and RhCMV are strong recapitulations of their cognate viruses in humans. Accordingly, rhesus macaques are the only available model to test the concept of CMV as a vaccine vector.

The SIV titration experiments are not designed for statistical significance. Instead, the number of animals for each inoculation titer (n=4) represents a compromise between achieving a minimal level of confidence of defining the minimal animal infectious dose and efficient use of the animal resource.

Similar arguments apply for the RhCMV pathogenesis monkeys. The goal is to determine whether the deleted variants can induce disease under conditions of severe immune deficiency. Previous work from our laboratory (Sequar *et al*, J. Virol. 76:7661, 2002) indicates that we should expect high levels of RhCMV replication and disease in the animals (2-3 per group of 4 animals). Failure to demonstrate replication or pathogenic potential in any of the animals of each group would constitute *prime facie* evidence of attenuation of virulence of the variant RhCMV. Performing the experiments in more animals would precisely define what level of attenuation was achieved. However, we feel that this is an issue best left to subsequent studies or accumulation of evidence over time. More importantly, it would not be a good use of the resource to include more animals initially if no attenuation was observed. Our goal is a clear demonstration of attenuated virulence. We believe that we can achieve this goal with the number of animals included. If necessary, additional animals will be added to the protocol

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?
Rhesus macaque	Ketamine	10	IM	As needed for anesthesia, based on the schedule of procedures outlined in section c. Anesthesia will be administered according to CNPRC SOP's.

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, neurologic deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

SIV will produce a severe and progressive immunodeficiency, leading to death. The time course for development of simian AIDS is variable but usually occurs within 6 months to 2 years. Clinical signs can include opportunistic infections, chronic diarrhea, weight loss, and anemia. CRPRC guidelines and recommendations will be followed for treatment modalities and euthanasia, to spare the animal unnecessary pain. Death is not an end-point. Animals in the titration experiments can be terminated once virological, immunological, and clinical parameters of infection establish that the animal has been infected. Animals involved in the pathogenesis of modified RhCMV variants may not develop clinical signs of simian AIDS by 6 months. Animals will still be terminated at 6 months to characterize the extent of RhCMV replication and histopathology.

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.

Signs of pain or discomfort will be treated according to CNPRC veterinary staff. Animals will be euthanized when necessary to spare the animal pain and discomfort.

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.

Euthanasia of animals will be done upon recommendation of CNPRC veterinary staff.

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?

10/02/02

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/internet	1964-2002	Rhesus cytomegalovirus; rhesus cytomegalovirus and SIV; RhCMV and vaccine; HCMV as vaccine vector
20 th Annual Symposium on Nonhuman Primate Models for AIDS abstract book	2002	Checked every abstract (n=136) for similar strategy as outlined in this proposal.

What were your findings with respect to alternative methodologies?

There are no alternative methodologies that elicit protective immune responses to HIV. The vaccine approach proposed herein has never been performed in 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?

All animals 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.

Species	Method	Drug	Dose (mg/kg)	route
Rhesus Macaque	overdose	Pentobarbital	60 mg/kg	IV

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

There will be no surplus animals.

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
CNPRC Director	Date	

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.

 nt).

[X] 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:

CNPRC Standard Operating Procedures will apply for all precautions and personal protective equipment.