

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

EH&S USE ONLY

PROTOCOL # 9588**EXPIRES: _____**

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

Species (common names):	Number:	Source:
Rhesus macaque	42	CRPRC

Project Title	Cytomegalovirus Infection and Inflammation		
Overnight housing location::	CRPRC	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.

Animals will be inoculated with wild type rhesus cytomegalovirus (RhCMV) by either the intravenous (IV) or intradermal (ID) routes of inoculation. Animals will also receive a one time ID injection of lipopolysaccharide (1 site) and saline (2 sites) either at the same time as RhCMV inoculation or 4 or 28 days post inoculation. Skin biopsies will be obtained at the ID sites at 2, 4, or 7 days post ID injection. Longitudinal blood draws will also be obtained.

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 infected with only RhCMV do not 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):	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: CRPRC

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 Primate Research Center (2-0447)
 VMTH Large Animal Field Service (2-0292) Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:
 Day phone:
 Emergency phone:
 Address:
 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: The goal of this proposal is to use the macaque model of HCMV to characterize the temporal relationship between acute inflammation and changes in parameters of RhCMV infection.

Hypothesis: It is hypothesized that human cytomegalovirus (HCMV) specifically targets activated endothelium at sites of inflammation for infection and replication. HCMV is a significant pathogen in individuals lacking a fully functional immune system, such as those infected with HIV, immunosuppressed transplant recipients, and fetuses. There is also evidence suggesting that chronic CMV infection in immunosuppressed hosts may be associated with development of atherosclerotic plaques. The mechanisms of viral pathogenesis are not well understood. Natural history and in vitro studies raise the possibility that HCMV exploits inflammation for viral dissemination and replication, particularly during rejection of solid organ allografts. Fulminant HCMV infection is frequently associated with acute rejection, and histologically occult infections may stimulate an immunopathological cascade. Such a scenario could result in unrestricted accumulation of host activated lymphoid cells within the allograft resulting in increased recognition of allogeneic tissue. To most microbial pathogens, areas of inflammation represent hostile environments. However, sequence analysis of the coding capacity of HCMV has led to the recognition that HCMV evolved the capacity to use host defense mechanisms for its own advantage. It is proposed that HCMV utilizes virally encoded β -chemokine receptors to enhance infection of activated endothelial cells.

Objectives: (1) Demonstrate that RhCMV targets inflammatory foci during acute viral infection. (2) Demonstrate that reactivated RhCMV localizes to inflammatory foci in long-term RhCMV seropositive animals.

Significance: Infection of rhesus macaques with RhCMV is an excellent model for HCMV persistence and pathogenesis. HCMV is strictly species-specific for infection, precluding infection of other species for analysis. There is increasing recognition that HCMV may localize to areas of inflammation and accelerate disease processes, particularly in solid organ transplant recipients. The rhesus macaque model we have designed is the only primate model available to conduct these key experiments.

b) Procedures employed in this project:

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

- | | | |
|---|---|--|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery surgical procedures | <input type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see 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.)*

Cutaneous inflammation: Published reports (· et al., 1994) have used intradermal (ID) injection of LPS to stimulate a focal area of inflammation. The authors report that rhesus macaques, like other non-human primates, are “relatively resistant to endotoxin administration”. They reported that 1.0 mg of LPS (derived from *Salmonella thyphosa*) in 0.1 ml of saline produced consistent inflammatory reactions in rhesus macaques. The inflammatory response was characterized by margination of polymorphonuclear leukocytes (PMN) by 2 hours post-treatment with a response that peaked at 24 hours when the PMN reaction became mixed with lymphocytes. Thereafter, lymphocytes were the dominant cell type up to 72 hours. A pilot study will be conducted to confirm the minimal dose of LPS necessary to elicit an inflammatory response. Two animals (6-12 months) will be used to establish inflammatory condition using an optimal concentration of LPS. Animals will receive three ID injections of LPS on the back at concentrations of 1.0, 0.5, and 0.25 mg in 0.1 ml saline. Animals will also receive a saline “sham” injection for a total of four injection sites. Skin biopsies will be performed at each injection site 24 hours post treatment. Tissue biopsies will be analyzed by a transplant pathologist for histological evidence of inflammation to determine the minimal dose of LPS required for induction of inflammation. Animals will be returned to the colony after completion of the pilot project.

RhCMV Inoculation: A total of 60 juvenile macaques (4-6 months) will be screened for seroreactivity to RhCMV. It is predicted that 40 of these will be seronegative for RhCMV, based on the epidemiology of RhCMV in the Primate Center macaque colony. 20 of the seronegative animals will be used for Group I. The predicted 20 seropositive animals will be used for Group II. The remaining 20 animals will be returned to the colony.

Group 1: Rhesus seronegative animals will be inoculated with wild-type RhCMV strain 68-1 and injected ID with LPS as follows.

Animals of two subgroups (1A and 1B, n=5 each subgroup) will receive 3 simultaneous ID injections consisting of either (a) RhCMV (1×10^5 PFU in 0.1 ml saline), (b) RhCMV + LPS (using the optimized LPS concentration determined above), or (c) saline. Injection sites will be located 10 cm apart on the back of each animal. Skin biopsies will be obtained from each injection site at either 2 (Group 1A) or 7 (Group 1B) days post injection and triaged for DNA purification (real-time PCR), formalin fixation (histology), and immunohistochemistry (snap-frozen in OCT). Blood draws will be obtained on the days of inoculation and skin biopsy, and processed for plasma and PBMC. Two additional subgroups of RhCMV seronegative animals (Groups 1C and 1D, n=5 animals each subgroup) will be inoculated intravenously with RhCMV (1×10^5 PFU in 1.0 ml saline). Animals will receive three ID injections of LPS/saline (1 site), and saline (2 sites) at either 4 (Group 1C) or 28 (Group 1D) days post RhCMV inoculation. Saline injection sites will be located on the back 5 and 25 cm distal to the LPS/saline injection site (also located on the back). Skin biopsies will be obtained 2 days post LPS/saline injection. Biopsy samples will be processed for DNA, histology, and immunohistochemistry. Blood draws will be obtained on the days of inoculation, LPS/saline treatment, and skin biopsy, and processed for plasma and PBMC.

Group 2: RhCMV seropositive animals will be subdivided into 4 subgroups (2A-2D, n=5 each subgroup). Animals of 2A and 2B will be treated with LPS only, and animals of 2C and 2D will be treated with LPS after a 7 day regimen of dexamethasone.

Animals of 2A and 2B (n=5 each) will each receive a single ID injection of LPS on the back and 2 sham (saline) injections, one located 5 cm and the other 25 cm distal to the LPS injection site. Skin biopsies will be performed at either 2 (2A) or 7 (2B) days post LPS/saline injection and processed identically to the biopsies described for Group 1. Blood draws will be obtained on the days of LPS/saline treatment and skin biopsy, and processed for plasma and PBMC. Animals of Groups 2C and 2D (n=5 each) will be treated with dexamethasone for 7 days (2 mg/kg once per day) prior to LPS treatment. After 7 days, each animal will receive a single ID injection of LPS on the back and 2 sham (saline) injections, one located 5 cm and the other 25 cm distal to the LPS injection site. Skin biopsies will be performed at either 2 (2C) or 7 (2D) days post LPS/saline injection and processed identically to the biopsies described for Group 1. Blood draws will be obtained on the days of dexamethasone initiation, LPS/saline treatment and skin biopsy. Animals will be slowly weaned from the dexamethasone treatment (every other day for a week, then every 4 days for 2 more doses) prior to returning the animals to the colony.

Animals will be fasted and sedated (ketamine) prior to all sample collections (skin biopsy, blood draws, virus inoculation, LPS/saline injection, dexamethasone treatment). Technicians and veterinarians at CRPRC will perform all animal handling, biopsies, and venipuncture.

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
Pilot Study	ID injection with LPS/saline, skin biopsy/ketamine	2	2
I	Confirm RhCMV seronegativity of juvenile macaques for (n=40). Return 20 of seronegative animals to CRPRC. RhCMV inoculation (intravenous or intradermal, n=10 each route), ID injections with LPS/saline, venous blood draws, skin biopsy, ketamine.	40	2
II	RhCMV seropositive animals (n=20), dexamethasone treatment (n=10), ID injections with LPS/saline, venous blood draws, skin biopsy, ketamine.	20	2

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?

Human cytomegalovirus (HCMV) is a significant pathogen in immunosuppressed individuals, such as those receiving solid organ allografts. There is epidemiological and in vitro evidence to suggest that HCMV may be a pathogenic component associated with inflammatory responses during allograft rejection. The rhesus macaque model is the most appropriate model to assess whether CMV targets areas of inflammation for infection and, thereby, accelerates disease outcomes.

This is a pilot project designed to gather data towards a larger study. Therefore, the number of animals may not be sufficient to achieve statistical significance. The number of animals of each subgroup represents the minimum number to obtain statistical significance, if the standard deviation of viral genome copy number on the skin biopsy is low. This number is based on our previous studies of RhCMV DNA loads in IV inoculated animals. Since these studies have never been conducted before, there are no prior variance estimates to accurately calculate minimum sample size.

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 mg/kg	IM	As needed for anesthesia, no more than once per day, according to CRPRC 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, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

RhCMV infection, experimental or natural, does not result in clinical signs of disease. Cutaneous administration of endotoxin (LPS) should result in a transient, focal inflammatory response that should resolve by 4 days with no long-term impact on animal well-being. Sites of inflammation should be examined for possible secondary bacterial infections. CRPRC guidelines and recommendations will be followed for treatment modalities and euthanasia.

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 CRPRC 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 CRPRC veterinary staff. Clinical signs will include excessive fluid and/or weight loss, failure to thrive, neurological impairment, and untreatable secondary infections.

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?

6/1/01

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

Database Name	Years Covered	Keywords / Search Strategy
PubMed	1964-2001	Rhesus cytomegalovirus; rhesus cytomegalovirus and inflammation; HCMV and inflammation
Internet		Rhesus cytomegalovirus; rhesus cytomegalovirus and inflammation; HCMV and inflammation

What were your findings with respect to alternative methodologies?

There are no alternative methodologies. This study cannot be performed in any other species, and it cannot be performed in tissue culture.

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 returned to the colony at the completion of the study.

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	Per CRPRC guidelines by CRPRC staff	Pentobarbital	60 mg/kg	IV

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

All animals will be returned to the colony at the completion of the study.

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
CRPRC Director	Date	

Committee Use Only Below

<p>** Conditions necessary for Committee Approval:</p>
<p>Final Disposition of this protocol:</p> <p>_____ Approved</p> <p>_____ Not Approved</p> <p>_____ Withdrawn by Investigator</p> <p>Date of Action: ____/____/____</p>

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
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Fitted Respirator

Type: _____

Other:

Describe: _____

- Personal protective equipment must be removed before leaving the room.
- Personal protective equipment must be discarded or decontaminated at the end of the project
- Hands, arms, and face must be thoroughly washed upon leaving the room
- Full shower, including washing of hair, must be taken upon leaving the room.
- Decontaminate Room (Inform ARS area supervisor when cage and/or room can be returned to general use).

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

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