

**PROTOCOL FOR ANIMAL USE AND CARE**Email to: [campusvet@ucdavis.edu](mailto:campusvet@ucdavis.edu)**CNPRC**

EH&amp;S USE ONLY

**PROTOCOL: 10651**  
**EXPIRES: 7/2/04**

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

| Species (common names): | Number: | Source: |
|-------------------------|---------|---------|
| Rhesus macaque          | 180     | CRPRC   |
|                         |         |         |
|                         |         |         |

|                                |  |                |  |
|--------------------------------|--|----------------|--|
| <b>Project Title</b>           | A Non-Human Primate Model for Cytomegalovirus Vaccines   |                |  |
| Overnight housing location::   | CRPRC  | Day use only : |  |
| Animals will be maintained by: | <input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator (If investigator maintained, attach husbandry SOP's.) |                |  |

**Procedures:** Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Animals will be immunized with multiple DNA expression plasmids for rhesus cytomegalovirus (RhCMV) proteins. After sufficient development of immune responses to the immunogens, animals will be challenged with RhCMV and prospectively analyzed for resistance to challenge.

**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): |       |

|                                |   |                                    |   |
|--------------------------------|---|------------------------------------|---|
| Funding source:                | NIH   | Previously approved?               | <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No |
| Is the project already funded? | <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No | Previous protocol number (if any): | 9100  |

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

|                          |  |                                     |   |
|--------------------------|--|-------------------------------------|---|
| <input type="checkbox"/> | Lab Animal Health Clinic ( 2-0514 )        | <input checked="" type="checkbox"/> | California Primate Research Center ( 2-0447 ) |
| <input type="checkbox"/> | VMTH Large Animal Field Service ( 2-0292 ) | <input type="checkbox"/>            | Another Veterinarian                          |

If you checked "Another Veterinarian", please provide:

|                  |  |          |  |
|------------------|--|----------|--|
| Veterinarian:    |  | Address: |  |
| Day phone:       |  |          |  |
| Emergency phone: |  | Email:   |  |

*If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email [pcstillman@ucdavis.edu](mailto:pcstillman@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 intent of this project is to test vaccination strategies that will protect from infection by human cytomegalovirus (HCMV). The proposal will use the rhesus macaque model of HCMV persistence and pathogenesis that my laboratory has developed.

**Hypothesis:** Human cytomegalovirus (HCMV) vaccines must be directed against both structural, regulatory, and immune modulating open reading frames (ORF) to reduce virologic parameters of infection and/or disease. Since disease potential is related to HCMV viral load, reductions in HCMV viral load will be enhanced when vaccination is directed against identified immunogens, such as glycoprotein B (gB) and phosphoprotein 65 (pp65), together with novel vaccine targets represented by viral regulatory and immune modulating ORF. This proposal will use the rhesus macaque model of HCMV to test the hypothesis.

**Objectives:** (1) Immunization of rhesus macaques with RhCMV structural proteins, including glycoprotein B (gB), and phosphoproteins (pp) pp65, pp28, and pp150, +/- viral regulatory and immune modulating proteins, such as immediate-early 1(IE1), UL144 (TNF- $\alpha$  receptor-like protein), IL-10. (2) RhCMV challenge of immunized animals.

**Significance:** Infection of rhesus macaques with RhCMV is an excellent model for HCMV persistence and pathogenesis. The vaccine strategy presented in this proposal is designed to disrupt those virus functions at the primary site of infection that both regulate viral gene expression and modulate local immune responses, facilitating HCMV replication and dissemination to distal sites of latency. The goal of vaccination is to attenuate the ability of HCMV to modify host immune responses, thereby enabling innate and adaptive immunity to contain the extent of viral replication. The hypothesis cannot be tested in non-primate models because rodent CMV do not encode the immune modulating ORF that form the basis of the immunization strategy.

**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 i 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.)

Group I: Juvenile macaques (4-24 months) (n=80) will be screened for seroreactivity to RhCMV by ELISA. Animals can be either male or female. Seronegative animals (n=40) will be selected for further study, and the remainder will be returned to the colony. Animals will be housed for up to 30 days segregated from RhCMV seropositive animals and multiple blood draws and saliva samples will be collected (every 14 days) to confirm maintenance of RhCMV seronegative status. Blood draws (1-10 ml) will be obtained from a peripheral vein and will not exceed the total amount permissible per month per animal (12 ml/kg/month). After 60 days, animals will be infected with RhCMV using a subcutaneous route of inoculation. Animals will be inoculated with  $10^6$ ,  $10^4$ ,  $10^2$ , or  $10^1$  plaque forming units (PFU) of wild-type RhCMV strain 68-1 (n=10 animals per titer). The viral inoculum will be delivered in a 100-microliter volume (tissue culture media as diluent). Animals will have blood draws and saliva/genital swabs at 0, 3, 5, 7, 10, 14 and 17 days, 3, 4, 6, 8, and 10 weeks post inoculation. After 10 weeks, the animals will be returned to the colony. The purpose of the animals in Group I is to establish the minimal animal infectious dose of RhCMV required to give 100% infection.

Group II: Juvenile macaques (4-24 months) (n=80) will be screened for seroreactivity to RhCMV by ELISA. Animals can be either male or female. Seronegative animals (n=40) will be selected for further study, and the remainder will be returned to the colony. Animals will be housed for up to 30 days segregated from RhCMV seropositive animals and multiple blood draws and saliva samples will be collected (every 14 days) to confirm maintenance of RhCMV seronegative status. Blood draws (1-10 ml) will be obtained from a peripheral vein and will not exceed the total amount permissible per month per animal (12 ml/kg/month). After 60 days, animals will be vaccinated intradermally (ID-abdomen) and intramuscularly (IM-triceps) with plasmid expression vectors (genetic immunization) for viral proteins. All of the animals will be immunized with gB- and pp65-expressing plasmids using needle delivery of plasmid (0.5 ml of plasmid into each triceps muscle and 3 x 0.1 ml of plasmid for the ID injections). DNA will be purified with endotoxin-free plasmid isolation kits (QIAGEN) and diluted in endotoxin-free saline. 150 micrograms of each plasmid will be used for IM immunizations and 50  $\mu$ g will be used for ID immunizations. 30 of the immunized monkeys will be immunized with an additional plasmid(s) for IE1 (n=10), UL144 + IL-10 (n=10), or pp28 + pp150 (n=10). Animals will be boosted with the same immunogens 4, 8, and 16 weeks after the initial vaccination. Multiple blood draws will be obtained prior to and during the immunization process. Blood draws will not exceed the total amount permissible per month for the size of the animal (12 ml/kg/month). Immunized animals will be prospectively

analyzed for immune responses. Approximately 6 months after the initial vaccination, animals will be challenged with wild type RhCMV (strain 68-1) by the subcutaneous inoculation of virus. The titer of challenge virus will be the minimal titer determined for Group I that infected 100% of the animals. The viral inoculum will be delivered in a 100-microliter volume (tissue culture media as diluent). Seronegative monkeys (n=10) will serve as naïve controls for virus inoculation. Animals will have blood draws at 0, 3, 5, 7, 10, 14, and 17, days, and 3, 4, 6, 8, 10, 12, 16, 20, and 24 weeks post challenge. Animals will be returned to the colony after 6 months.

Group III: Juvenile macaques (4-6 months) (n=20) will be screened for seroreactivity to RhCMV by ELISA. Animals can be either male or female. Seronegative animals (n=10) will be selected for further study, and the remainder will be returned to the colony. Animals will be housed for up to 30 days segregated from RhCMV seropositive animals and multiple blood draws and saliva samples will be collected (every 14 days) to confirm maintenance of RhCMV seronegative status. Blood draws (1-10 ml) will be obtained from a peripheral vein and will not exceed the total amount permissible per month per animal (12 ml/kg/month). After 30 days, animals will be infected with RhCMV using the subcutaneous route of inoculation. These monkeys will serve as unimmunized controls. Animals will be inoculated with the minimal animal infectious dose (determined from Group I animals) of RhCMV strain 68-1 (n=10) by the subcutaneous route. The viral inoculum will be delivered in a 100-microliter volume (tissue culture media as diluent). Animals will have blood draws and saliva/genital swabs at 0, 3, 5, 7, 10, 14, and 17, days, and 3, 4, 6, 8, 10, 12, 16, 20, and 24 weeks post challenge. Animals will be returned to the colony after 6 months.

A skin punch biopsy (6 mm) will be obtained from each animal in Groups I-III prior to RhCMV inoculation to start autologous fibroblast cultures. An axillary lymph node biopsy will be obtained from each virus inoculated animal (1-4 weeks post inoculation; optimal time to be determined from ongoing studies) of Groups I-III. LN biopsies will be performed just one time per monkey. Animals will be fasted and sedated (ketamine) prior to all sample collections. Pain medication (oxymorphone) may be used as a post biopsy analgesic, based on discretion of CNPRC veterinary staff. Technicians and veterinarians at CNPRC 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 |
|-------|--|-------------------|----------|
| I     | RhCMV Inoculation (subcutaneous), venous blood draws, oral/genital swab, lymph node biopsy/ketamine for sedation; oxymorphone for analgesic, as needed       | 80                | 2        |
| II    | RhCMV Inoculation (subcutaneous), venous blood draws, oral/genital swab, skin punch biopsy, lymph node biopsy/ketamine, oxymorphone for analgesic, as needed | 80                | 2        |
| III   | RhCMV Inoculation (subcutaneous), venous blood draws, oral/genital swab, skin punch biopsy, lymph node biopsy/ketamine, oxymorphone for analgesic, as needed | 20                | 2        |
|       |  |                   |          |

## Categories of invasiveness

| Category | Description  |
|----------|--|
| 1        | Little or no discomfort or stress<br><b>Examples:</b> 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<br><b>Examples:</b> 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<br><b>Examples:</b> 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<br><b>Examples:</b> 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?

Cytomegalovirus (CMV) is a significant pathogen in immunosuppressed individuals, such as those infected with HIV or in transplant recipients. Host immune responses to CMV are important for controlling virus replication and limiting CMV disease. The rhesus macaque model is the most relevant animal model to assess mechanisms of CMV persistence and pathogenesis in immunocompetent and immunodeficient hosts. Accordingly, the rhesus macaque model is the most appropriate model to investigate vaccine strategies under in vivo conditions that most closely resemble the human condition.

The number of animals for Group I is minimum number required to determine the minimum titer of RhCMV required to infect 100% of animals. Our goal is to (1) characterize the parameters of viral infection by the route of inoculation at different titers of inoculation, and (2) determine the minimal animal infectious dose for subcutaneous inoculation.

The number of animals per group for Groups II-III (n=10 each vaccination regimen) is based on the need to ensure that we can distinguish statistically significant as well as biologically meaningful differences between the different vaccine groups. Reductions in plasma viral load following challenge will be used to assess changes in the in vivo growth parameters following the different vaccine immunogens.

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  | Ketamine | 10 mg/kg     | IM    | As needed for                        |

|                |             |                 |    |   |
|----------------|-------------|-----------------|----|---|
| macaque        |             |                 |    | anesthesia, no more than once per day, according to CNPRC SOP's.  |
| Rhesus macaque | Oxymorphone | 0.01-0.15 mg/kg | IM | As needed for postprocedure analgesics, 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, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

None adverse effects are expected from either RhCMV inoculation or genetic immunization, based on our extensive prior experience with both procedures. The lymph biopsy will require a small incision (under anesthesia). This will result in a localized pain and tenderness that should subside within a few days. Animals will be treated, as needed, with analgesics to alleviate any pain and discomfort (Oxymorphone, 0.01-0.15 mg/kg, IM). CNPRC guidelines and recommendations will be followed for treatment modalities and euthanasia, in the highly unlikely event that there is a serious adverse effect.

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.

CNPRC guidelines and recommendations will be followed for treatment modalities and euthanasia, if necessary. Animals will be treated, as needed, with analgesics to alleviate any pain and discomfort (Oxymorphone, 0.01-0.15 mg/kg, IM).

*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.*

**j) Literature search for alternatives and unnecessary duplication:**

*Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. "Alternative methodologies" refers to reduction, replacement, and refinement (the three*

*R's) of animal use, not just animal replacement. You must also show that this use of animals is not unnecessarily duplicative of other studies.*

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

*[http://trc.ucdavis.edu/jawelsh/Databases\\_Med\\_Vet\\_Researchers.htm](http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm) (email: [jawelsh@ucdavis.edu](mailto:jawelsh@ucdavis.edu))*

*or [http://www.vetmed.ucdavis.edu/Animal\\_Alternatives/main.htm](http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm) (email: [mwood@ucdavis.edu](mailto:mwood@ucdavis.edu))*

What was the date on which you conducted this search?

5/30/03

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 - present | Cytomegalovirus and vaccine |
| ISI Web of Science | 1975 - present | Cytomegalovirus and vaccine |
|                    |                |                             |
|                    |                |                             |

What were your findings with respect to alternative methodologies?

This type of study has never been performed in primates. No one outside of my laboratory is in a position to conduct this study in non-human primates, and genetic immunization has not been performed in humans for cytomegalovirus.

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 CNPRC. Euthanasia of animals, if necessary, will be done upon recommendation of CNPRC veterinary staff. Clinical signs will include excessive fluid and/or weight loss, failure to thrive, neurological impairment, and untreatable secondary infections

**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?

All animals can and will be returned to the CNPRC for use in other studies. Since RhCMV infection is endemic in CNPRC macaques, experimental inoculation with RhCMV does not introduce a novel or exotic pathogen into the population.





**ANIMAL ROOM SAFETY INFORMATION**

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

**PROTOCOL #** 10651**EXPIRES:** \_\_\_\_\_

|  |
|--|
|  |
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RUA#: \_\_\_\_\_

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BUA#: 0609

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CCA#: \_\_\_\_\_

|  |
|--|
|  |
|--|

Identity of Hazard: Rhesus cytomegalovirus (RhCMV)

Investigator Last Name: \_\_\_\_\_ Dept.: \_\_\_\_\_

First Name: \_\_\_\_\_ Phone: \_\_\_\_\_

Email: \_\_\_\_\_ Fax: \_\_\_\_\_

**Provide a short description of the agent:**

RhCMV is a member of the herpesvirus family of viruses and is a ubiquitous infectious agent in colony-reared macaques.

This agent / material is hazardous for:  Humans only  Animals only  Humans and Animals  
For which Animal Species?The agent can be spread by:  Blood  Feces/urine  
 Saliva/nasal droplets  Does not leave animal  
 Other:**Describe any human health risk associated with this agent:**

RhCMV is an endemic infectious agent in rhesus macaques. Immunocompetent macaques display no clinical signs of infection with RhCMV, although RhCMV establishes a persistent infection for the life of the host. RhCMV is a serious cause of morbidity and mortality in immunosuppressed macaques, including those infected with SIV. In addition, RhCMV can cause serious neuropathologic outcomes in experimentally infected macaque fetuses. It is not known whether RhCMV can infect a human host. There have been no reported pathologic consequences associated with RhCMV infection in humans. However, RhCMV readily infects cells of human origin in tissue culture. Because of this, the virus should be regarded as a potential human pathogen.

**The precautions checked below apply to this experiment:**

- The researcher or his/her technicians are responsible for the feeding and care of these animals.  
 The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians.
- Cage  Stall  Water Bottle  Animal Carcasses  
 Bedding  Other:
- Cages must be autoclaved before cleaning.  
 Label cages and remove label after decontamination.  
 Animal carcasses must be labeled and disposed of as follows:  
 Incineration  Biohazardous Waste Container  
 Bag and Autoclave  EH&S will pick-up (2-1493).  
 All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:  
 Incineration  Biohazardous Waste Container  
 Bag and Autoclave  EH&S will pick-up (2-1493).

**Personal Protective Equipment Required:**

- The following personal protective equipment must be worn/used in the room:
- Lab Coat/Coveralls  Shoe Covers/Booties  
 Disposable Gloves  Head Cover  
 NIOSH Certified Dust Mask  Disinfectant footbath  
 Eye Protection/Face Shield   
 Fitted Respirator Type:  
 Other: Describe:

- 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:**

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

6/5/2003

Pre review questions protocol 10651

Hi ,

I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10651 for future reference. I have attached a copy of the protocol for ease of making revisions.

For this protocol to be considered on the committee agenda of June 19th, please return your revised protocol to me on or before noon, Tuesday June 10th.

Thank you in advance,

Protocol 10651 ( )

1. In section c, you discuss blood collection in multiple places, but do not include the blood volume. Please expand to include the volume of blood and location used to collect the blood.

2. In group II, you state that 30 of the 40 animals will be boosted. What happens to the other 10? Please clarify. Also, what volume of vaccine challenge will be administered subcutaneously after 6 months?

3. In section c, group III, you state that animals will be infected with RhCMV using the subcutaneous route. What volume of agent plus vehicle will be administered? Please clarify.

4. In section j, you state that you do not expect any adverse effects, yet you will be taking biopsies. Please address the possible pain from biopsies and post procedural analgesics used.

5. In your literature search, you have listed a symposium for your second database. USDA will not longer anything but databases, so please include a second database literature search.

6. In section l, you have listed the CNPRC guidelines, but the committee needs the information spelled out on the protocol, so please include the method of euthanasia, the agent, dose and route.

Date: Wed, 02 Jul 2003 07:20:07 -0700  
To:  
From:  
Subject: Fwd: Re: committee questions

Date: Tue, 01 Jul 2003 18:33:40 -0700  
Subject: Re: committee questions  
From:  
To:

>>> #10651(       ):

>> 1. How do they know that 10 animals per group will ensure statistical significance? Please clarify.

The number of animals per group for (n=10) is based on the need to ensure that we can distinguish statistically significant as well as biologically meaningful differences between infection with wild-type RhCMV between vaccinated animals and unvaccinated controls. Reductions in plasma viral load following challenge will be used to assess changes in the in vivo growth parameters of the vaccinees. Minimum sample size was calculated according to the InStat software (v.2.03, GraphPad Software, Inc.) using prior variance estimates. Numerous studies of ours (>30 monkeys) quantifying RhCMV DNA in plasma at 1 week post IV inoculation indicate that there can be approximately 10-fold differences in plasma viral load (standard deviation = 0.5 logs). We have also observed that immune responses to prior infection with RhCMV significantly reduce plasma viral loads 1 week after challenge inoculation with RhCMV. We predict that differences between the vaccinees and controls will be large. We are making the conservative assumption that variance will be larger (1.1 - 1.5 logs) in a larger group of animals. Based on this assumption, group sizes of 10 each are required to provide sufficient statistical power (0.80,  $\alpha=0.05$ ) to detect a minimum 1.5 - 2 log differences in plasma DNA load. If the standard deviation for each group is 1.1 logs, the minimum difference detected with inoculation groups of 10 would be 1.5 logs (31-fold). If the standard deviation for each group is 1.5 logs, only differences greater than 100-fold would be considered significant. Statistical power to detect differences of lesser magnitude is reduced, if the standard deviations are within this range. If standard deviations are less than 1 log, smaller differences in viral load would be considered significant.

Sincerely,