PROTOCOL FOR ANIMAL USE AND CARE

Email to: campusvet@ucdavis.edu

 investigator

Last Name: [ ] Call Investigator
First: [x] Call Investigator
Middle: [ ] Call Investigator
email: [x] Call Investigator
Department: [ ] Call Investigator
Phone / Fax: [x] Call Investigator
After hrs. #: [ ] Call Investigator

Species (common names): Cynomolgus macaque
Number: 14
Source: CNPRC

Project Title: Production of Pedigreed SPF Rhesus Macaque

Overnight housing location: CNPRC
Day use only: [x] Investigator
Animals will be maintained by: [x] Investigator (If investigator maintained, attach husbandry SOP's.)

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

The intent of the project is to evaluate the immunogenicity and efficacy of a novel vaccine against Coccidioides immitis, the agent of Valley Fever.

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 be housed in infectious housing for the infectious challenge portion of the study.

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

Sick Animals
[ ] Call Investigator
[x] Clinician to treat
[ ] Terminate
[x] Necropsy

Dead Animals
[ ] Call Investigator
[ ] Save for Investigator
[ ] Bag for disposal
[x] Necropsy

Pest Control
[x] Call Investigator
[ ] OK to use pesticides
[ ] No Pesticides in animal area

Hazardous Materials (only if in the animal room):

Infectious Agents? [x] Yes [ ] No
Agent(s): Coccidioides immitis

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

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

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

Coccidioidomycosis (Valley Fever) is a potentially severe fungal infection that is increasing in incidence in endemic areas of the US. The etiologic agent, C. immitis, is a dimorphic fungus naturally found in soil. Infection of humans and animals occurs through inhalation of infectious arthrospores produced by the soil phase of the fungus. An effective vaccine against this disease would greatly reduce the health and economic burdens on human populations in endemic areas. Adult cynomolgus macaques will be immunized with a novel vaccine construct plus adjuvant previously demonstrating efficacy in a mouse model, and monitored for immune response. If immune response is of sufficient magnitude, immunized animals will be challenged with infectious arthrospores to determine level of protection.

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.
- Aversive conditioning.
- Special diets; food or water treatment.
- Induced illness, intoxication, or disease
- Death as an endpoint (see i below)
- Trapping, banding or marking wild animals

** 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.)*

Adult female cynomolgus macaques (n=14) will be randomly assigned to one of three groups, and will receive a series of 3 IM injections of prototype vaccine and/or adjuvant 3-4 wks apart. Group 1 animals (n=5) will receive a high dose antigen preparation (5 ug) plus adjuvant. Group 2 animals (n=5) will receive a low dose antigen preparation (1 ug) plus adjuvant. Group 3 animals (n=4) will receive adjuvant only. Adjuvants to be used will be ISS (a CpG oligonucleotide; Dynavax) or Montanide. ISS may not be available to us due to licensing and materials transfer issues that have yet to be resolved. If ISS is not available, the study will be done using Montanide. Blood samples (6 ml) will be obtained via femoral venipuncture prior to immunization (day 0) and 14 days after each subsequent immunization, for CBC, antigen-specific in vitro immunologic assays, mRNA extraction, and reference Serum/plasma. Blood samples will be collected with animals under ketamine anesthesia (10mg/kg IM). If immune responses to the antigens are determined to be of sufficient magnitude, animals will be challenged by intratracheal administration of infectious arthrospores (2500 arthrospores in 1.0 ml PBS). Inoculation procedure will be performed under BSL-3 conditions. Bronchoalveolar lavage (BAL) and blood samples (3 ml) will be collected for pre-challenge baseline immunologic assays, and on post-challenge days 3, 7 and 10. BAL and blood collections will be performed with animals under Telazol (mg/kg IM) anesthesia. A physical examination will be performed and thoracic radiographs, reference serum samples, and body weights will be obtained prior to challenge, and at 3 week intervals for 60 days post challenge to monitor clinical outcomes.

d) **Study Groups and Numbers:** Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; you may add as many rows as you require. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vaccine High Dose</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Vaccine Low Dose</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Adjuvant only controls</td>
<td>4</td>
<td>2</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&lt;br&gt;&lt;br&gt;&lt;strong&gt;Examples:&lt;/strong&gt; 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&lt;br&gt;&lt;br&gt;&lt;strong&gt;Examples:&lt;/strong&gt; 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&lt;br&gt;&lt;br&gt;&lt;strong&gt;Examples:&lt;/strong&gt; 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&lt;br&gt;&lt;br&gt;&lt;strong&gt;Examples:&lt;/strong&gt; 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 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?

We have previously demonstrated that <i>Macaca fascicularis</i> is a suitable experimental pathogenesis model for coccidioidomycosis, and that the range of responses of individual animals to infection can be adequately addressed with 5 animals per group.

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>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Who will be the surgeon?

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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>Cynomolgus</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>For blood sampling and physical examination</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>Telazol</td>
<td>5</td>
<td>IM</td>
<td>For intratracheal inoculation and BAL</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)

Little in the way of adverse effects are expected in the immunization phase of the study. Adjuvants and antigens used for immunization have been well tolerated in mice, and these adjuvants have been safe and well tolerated in humans and nonhuman primates.

Adverse effects associated with Cocci challenge include induction of mild to severe respiratory disease with granulomatous pneumonia, and potential systemic infection affecting other organs including bone, CNS, heart, liver.

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.

Supportive and specific veterinary care will be provided at the discretion of primate center veterinarians, including use of analgesics as deemed appropriate. Animals developing severe disease unresponsive to supportive therapy will be euthanized.

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.

Death is not an endpoint of this study. Animals developing severe disease unresponsive to appropriate therapy 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 (email: jawelsh@ucdavis.edu)

or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwwood@ucdavis.edu)

What was the date on which you conducted this search? [ ] June 10, 2003

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>1975-2003</td>
<td>Macaca fascicularis, Coccidioides immitis, Valley Fever, Coccidioidomycosis, animal models, nonhuman primates, vaccine</td>
</tr>
<tr>
<td>BioMedNet</td>
<td>1975-2003</td>
<td>Macaca fascicularis, Coccidioides immitis, Valley Fever, Coccidioidomycosis, animal models, nonhuman primates, vaccine</td>
</tr>
</tbody>
</table>
What were your findings with respect to alternative methodologies?

Literature from the 1970’s indicates cynos are a suitable animal model system for C. immitis pathogenesis, as the same spectrum of disease is observed naturally and experimentally infected macaques as in infected humans. There are no alternatives to use of live animals for vaccine efficacy evaluation. The antigen construct we are using has shown promise in protection from C. immitis-induced disease in a mouse model, and confirmation of efficacy in a nonhuman primate model will facilitate advancement of candidate immunogens to human Phase I trials.

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.

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

Animals developing severe disease unresponsive to specific or supportive treatment during the course of the study will be euthanized. All animals will be euthanized at the conclusion of the study for histologic examination of various tissues.

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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<tbody>
<tr>
<td>Cynomolgus</td>
<td>Drug overdose</td>
<td>Pentobarb</td>
<td>60</td>
<td>IV</td>
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</table>

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

All animals will be euthanized at the conclusion of the challenge phase of the study.
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://ehs.ucdavis.edu/animal/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. Information is available on the world wide web at http://ehs.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/](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.

---

**Adjunct Professor**       | **Principal Investigator**
---                         | **Rank / Title**
**6/30/03**                | **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.

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

Identity of Hazard: Coccidioides immitis

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

Provide a short description of the agent:
Coccidioides immitis is a dimorphic fungus. Only arthrospores produced in the vegetative phase (in soil or culture medium) are infectious. No arthrospores are produced in infected animals. Once infected, animals are non-infectious to humans or other animals by routine contact.

This agent / material is hazardous for: [ ] Humans only [ ] Animals only [x] Humans and Animals
For which Animal Species?

The agent can be spread by: [ ] Blood [ ] Feces/urine
[ ] Saliva/nasal droplets [ ] Does not leave animal
[x] Arthrospores produced only in culture or in soil

Describe any human health risk associated with this agent:
C. immitis is the causative agent of coccidioidomycosis (Valley Fever). Infection results from inhalation of infectious arthrospores produced only in soil or culture medium. Infection results in mild to severe respiratory disease, with fever, flu-like symptoms, and pneumonia. Infected animals are NOT infectious via aerosol or contact exposure. Human exposure may occur through accidental inoculation with infected tissues (e.g. cuts) at necropsy.

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 [x] Animal Carcasses
[ ] Bedding [ ] Other:

[ ] Cages must be autoclaved before cleaning.
[ ] Label cages and remove label after decontamination.
[x] Animal carcases must be labeled and disposed of as follows:

[ ] Incineration [x] 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:

[x] Lab Coat/Coveralls [x] Shoe Covers/Booties
[x] Disposable Gloves [x] Head Cover
[ ] NIOSH Certified Dust Mask [ ] Disinfectant footbath
[ ] Eye Protection/Face Shield [ ]
[x] Fitted Respirator Type: Positive pressure PAPR unit – ONLY for inoculation
[ ] Other: Describe:

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

Provide any other information needed to safely work in this room:
Only the inoculation procedure is associated with risk of human exposure. Inoculation procedure will be performed by investigator under BSL-3 guidelines. Once inoculated and infected, animals are not infectious.
Date: Mon, 21 Jul 2003 13:16:16 -0700
To:
From:
Subject: Fwd: Re: pre review questions protocol 10735

Reply-To:
From:
To:
Subject: Re: pre review questions protocol 10735
Date: Mon, 21 Jul 2003 11:46:46 -0700

Below are my answers to the questions, and attached is a revised/correct protocol

1. ISS is the adjuvant of choice, but this may not be available to use due to licensing and materials transfer issues that are not yet resolved. If ISS is not available, the study will be performed using Montanide.

2. Blood sample will be collected at 14 days post-immunization.

3. Physical exams and radiographs will be performed at 3 week intervals for 60 days post-challenge.

4. Should read 60 mg/kg for pentobarbital

5. Not sure exactly what is missing - although name and contact info were not filled in at the top. Once infected, these animals are not infectious. The infectious arthrospores of this fungus are produced in soil (or culture medium) but NOT in infected animals. There is no animal to animal (or animal to human) transmission. Carcasses of infected animals should be disposed of as biohazardous, but the actual inoculation procedure with infectious arthrospores is the only significant risk for human exposure/infection. Infected animals will not be the source of environmental contamination within the animal room.

----- Original Message ----- 
From: " 
To: 
Sent: Monday, July 21, 2003 11:04 AM 
Subject: Fwd: pre review questions protocol 10735 

> I still need answers to these protocol questions. It's supposed to go to the meeting this week!
> 
> >
> >Hi ,
> >
> >I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10735 for future reference. Since I have some questions, I have attached a copy of the protocol with the number embedded for ease of making revisions to the questions below.
> >
> >For this protocol to be considered on the committee agenda of July 31, please forward your revised protocol to me on or before noon, Tuesday, July 22.
> >
> >If you have any questions, feel free to contact me via phone or email.
> >
> >thanks in advance,
> >
> >
> >Protocol 10735 ( )
> >

University of California, Davis
Printed 7/21/2004 10:41:32 AM Page 10
1. In section c, you mention using two adjuvants, but do not qualify which is used when. Please explain what determines which one is used for which groups if you can.

2. In section c, you state that you will collect blood at day 0 and 10-14 days after each subsequent immunization. What determines when you will collect the subsequent samples? Is an approximate time between 10-14 days adequate?

3. In the last sentence of section c, you state you will perform a physical examination at 3 week intervals. How long will you perform the physical examinations? Months or years? Then what happens? Please clarify.

4. In section l, you have listed the dose of pentobarbital as 100mg/kg when the CNPRC vet staff use 60 mg/kg. Is there a reason why you will be using a different dose?

5. In the animal room safety information page, the information at the top of the page was omitted and some of the boxes under "precautions" appeared to be incomplete. Please review and complete the form.