

PROTOCOL FOR ANIMAL USE AND CAREEmail to: campusvet@ucdavis.edu**CNPRC**

EH&S USE ONLY

PROTOCOL: 10829
EXPIRES:

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

Species (common names):	Number:	Source:
Rhesus Macaque	30	CRPRC

Project Title	Anti Tumor Necrosis Factor Therapy in Simian AIDS		
Overnight housing location::	CRPRC	Day use:	
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.

This project proposes to determine the effects of anti Tumor Necrosis Factor therapy in addition to antiretroviral therapy, during the primary as well as asymptomatic stages of virus infection in SIV infected rhesus macaques. Procedures include SIV infection, intestinal and lymph node biopsy, blood collection, RDP58 and PMPA administration, intestinal and lung lavages, and jejunal and colonic aspirates, D-xylose absorption test.

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.

These animals will be housed in infectious housing after being infected with **SIV**mac251.

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 type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input 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 checked="" 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
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:	National Institutes of Health	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):	9228

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 pctlillman@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.

The goal of this project is to determine the anti-inflammatory effect of RDP58 (a translational inhibitor of Tumor Necrosis Factor, TNF) administration in conjunction with PMPA(9-[2-(phosphonomethoxy)ethyl] adenine) therapy on the treatment of intestinal disease and inflammation caused by SIV infection in rhesus macaques. RDP58 is a novel TNF inhibitor that can suppress clinical and pathologic complications in mouse and monkey colitis models following oral administration. Gastrointestinal disease accompanied by inflammation is seen in HIV infected patients. SIV-infected rhesus macaque is a valuable animal model for the study of intestinal disease in AIDS. Macaques in the primary and asymptomatic stages of SIV infection will be treated with RDP58 and PMPA, and the effects on TNF levels, inflammation, viral replication and nutrient absorption will be examined. Most studies examining suppression of TNF expression and its effect on the immune response are being performed in rodents or macaques suffering from inflammatory bowel disease. Similar gut associated complications exist in HIV infected humans and SIV infected macaques. The gut associated lymphoid tissue (GALT) harbors greater than 85% of the lymphoid tissue in the body. It is the primary site of rapid viral replication and dissemination in early stages of infection. Thus evaluation of gut and peripheral tissues will be critical in determining the true efficacy of anti-TNF therapy and the immune response to early SIV infection. We propose to examine lymphocytes and immune cells from intestinal lymphoid tissue as well as cells in blood and other lymphoid organs and compare them to untreated control animals. We will examine immunophenotypic changes, cytokine expression, and viral loads in these cells following anti-TNF therapy in acutely infected animals to determine the efficacy of anti-TNF therapy at the whole animal level. Studies of this nature are not yet feasible in HIV-infected patients during antiretroviral therapy. The proposed studies will be valuable in determining whether early immune intervention affects the clinical, immunologic and virologic outcome of SIV infection.

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 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 checked="" type="checkbox"/> endoscopy |
| <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.)

A total of 30 animals will be used in this study. Sixteen animals will be infected intravenously (1000 TCID₅₀) with pathogenic SIVmac251. The RDP58 (anti-TNF) treatment (2mg/Kg/day/orally) and PMPA will be initiated in 6 animals at 2 weeks post-infection and 6 animals at 14 weeks post infection. PMPA treatment 30mg/Kg/day, subcutaneously. After 3 months at 30mg/Kg/day PMPA (subcutaneously), animals will have monthly chemistry panels, performed by the CRPRC Clinical Lab, to determine possible PMPA toxicity. If toxicity occurs in an animal receiving PMPA at 30mg/Kg/day, PMPA dosage will drop to 20mg/kg/day (sub-Q) until time of necropsy. PMPA is a nucleotide analogue with potent anti-retroviral activity. Six animals will serve as uninfected normal controls. Six uninfected animals will be administered with PMPA for 20 weeks which will serve as uninfected treated controls. Animals will be weighed weekly and monitored daily by CRPRC staff. RDP58 will be given on a daily basis for 20 weeks at 2 mg/Kg dosage by oral route. Blood samples (5 to 10 mls) will be collected at designated time points (pre-infection, 2, 4, 8, 10, 14, 20 weeks post-infection) for evaluation. Lymph node and jejunal biopsies (using endoscopy) will be obtained at various time points (pre-infection, 2, 8, 14, 20 weeks post infection). Six animals with SIVmac251 infection only for 34 weeks and serve as SIV infected control animals. After 2 weeks of SIVmac251 infection. Six animals will receive PMPA and RDP58 for 20 weeks. And after 14 weeks of SIVmac251 infection, Six animals will receive daily PMPA and RDP58 for 20 weeks. Each SIV infected group will be weighed weekly and monitored daily by CRPRC staff. PMPA and RDP58 will be given, at the designated post-infection timepoints, on a daily basis for 20 weeks at, respectively, 20mgs/Kg/day sub cutaneously and 2 mg/Kg dosage by oral route. Blood samples (5 to 10 mls) will be collected at designated time points (pre-infection, 2, 4, 8, 10, 14, 20 weeks post-infection) for evaluation. Lymph node and jejunal biopsies (using endoscopy) will be obtained at various time points (pre-infection, 2, 8, 14, 20 weeks post infection)

Jejunal pinch biopsies will consist of 12 small (20-25 mg each) tissue pieces from which 4 to 6 million cells can be isolated. Briefly: animals, after overnight fasting, will be sedated using Ketamine and/or Telazol. A pediatric endoscope will be utilized to perform the endoscopy procedure. The edoscopy tube is inserted into the mouth and subsequently passed into the esophagus, the stomach and the past the pyloric sphincter and into the upper small intestine. Each pinch biopsy sample is take by a small instrument that threads through the endoscopy tube. Each biopsy sample will be placed into cold buffer (provided in advance from the laboratory). There is the risk of intestinal perforation with jejunal biopsies. When a perforation occurs, a jejunal resection will be performed. Briefly: Animals will be anesthetized with Telazol. A ventral midline abdominal incision will be made. The jejunum would be exteriorized and clamped to preserve the vascularity with Doyen forceps. An approximate 3-5 cm section of jejunum would be removed and an anastomosis would be performed using absorbable suture. The abdomen would be lavaged and then closed routinely in 3 layers. Antibiotics are given afterwards: enrofloxacin (5mg/Kg/day for 10 days) and metronidazol (50mg/Kg orally/day for 10 days).

Animals will be euthanized at 22-34 weeks post-infection according to the CRPRC guidelines. A complete necropsy will be performed for each animal and tissue and blood samples will be collected. Peripheral and systemic lymphoid tissue derived cells will be prepared for histological, immunohistochemical, flow cytometric and virologic analysis.

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
1	After 2 weeks of SIVmac251 infection, animals will receive PMPA and RDP58 in sugar water daily for 20 weeks, jejunal and lymph node biopsy and blood samples, D-xylose absorption .	6	3
2	After 14 weeks of SIVmac251 infection, daily PMPA and RDP58 administered orally in sugar water for 20 weeks, jejunal and lymph node biopsy, blood collection, D-xylose .	6	3
3	6 animals with no viral infection. sugar water for 20 weeks. Perform jejunal and lymph node biopsy, blood collection, D-xylose absorption .	6	3
4	6 animals with SIVmac251 infection only for 34 weeks, jejunal and lymph node biopsy, blood collection, D-xylose absorption .	6	3

5	6 uninfected animals will receive PMPA for 20 weeks, jejunal and lymph node biopsy, blood collection, D-xylose absorption.	6	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 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?

Intestinal abnormalities including nutrient malabsorption, diarrhea and wasting are common features of HIV-1 infection. Studies on the effect of anti-TNF therapy on HIV associated enteropathy are limited due to difficulties in obtaining sufficient amounts of intestinal tissues for analysis at different time points following viral infection and therapy. SIV infected rhesus macaques are extremely valuable as a suitable animal model to examine the effect of RDP58 and PMPA therapy on viral replication and the immune modulation and function in gut associated lymphoid tissues of SIV infected rhesus macaques. Many studies have demonstrated that the immunophenotypic and functional alterations occurring in intestinal tissues following SIV infection do not parallel those seen in the peripheral blood. Thus the effects of anti-TNF therapy on T cell dynamics in the gastrointestinal lymphoid tissue and lymphoid tissues at other sites independent of peripheral blood warrants examination in order to determine the true efficacy of RDP58 therapy.

SIV infected macaques are the most relevant animal model to study the pathogenesis of HIV-1 associated enteropathy. There is no comparable lentivirus infection animal model available that is suitable for the studies of pathologic and functional alterations in intestinal epithelial and lymphoid populations during the course of disease development.

Thirty animals will be used for this study. Of these, eighteen will be infected with pathogenic SIVmac251 covering the acute (2 week) and chronic (14 week) infection and treated daily with RDP58 and PMPA until necropsy. Six animals will serve as uninfected controls. Six uninfected animals will receive PMPA and will serve as treated controls. Animals will be euthanized at 22 to 34 weeks post SIV infection. Due to animal to animal variation, it is necessary to have a minimum of 3 to 6 animals in each group in order to obtain statistically relevant data.

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?
M. mulatta	telazol	5 mg/kg	IM	before biopsy procedure
M. mulatta	medetomidine	(30 ug/Kg)	IM	prior to lymph node biopsies
M. mulatta	Buprenorphine	0.01-0.03 mg/kg	IM	BID for 3 days, discretion of CRPRC vets
M. mulatta	enrofloxacin	5mg/Kg/day	IM	for 10 days after jejunal biopsies
M. mulatta	Ketamine	10mg/Kg/day	IM	before biopsy procedure or blood collection
M. mulatta	Ketoprophen	2mg/Kg/day	IM	for 3 days after lymph node biopsies
M. mulatta	atipamezole	0.15 mg/Kg	IM	prior to lymph node biopsies
M. mulatta	metronidazol	50mg/Kg/day	orally	for 10 days after jejunal biopsies

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)

Discomfort may accompany intestinal biopsies, however animals are anesthetized during the entire procedure. Intestinal biopsies may lead to intestinal perforation, bleeding and death.

Blood collection may be associated with minimal discomfort.

Long term PMPA use can be toxic by causing bone loss. There is no known toxicity due to combining PMPA and RDP58.

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.

Analgesics or any post-operative procedures may be utilized as deemed necessary by the attending veterinarian.

Animals will be euthanized according to CRPRC criteria for euthanasia of SIV infected macaques. This would include weight loss of >15% in 2 weeks, persistent leukopenia, total WBC<3,000, opportunistic infections that do not respond to therapy, dehydration >7% and not responsive to oral hydration therapy for 3 days, lymphopenia, abdominal lesions and severe depression (obtusion).

Bone loss due to PMPA administration will be treated with supplements as per veterinarian's request.

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 (email: jawelsh@ucdavis.edu)

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

What was the date on which you conducted this search?

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
Current Contents	Sept 1995-2003	AIDS, SIV infection, intestine, PMPA, TNFalpha inhibitors
Pub Med	Sept 1995-2003	AIDS, SIV infection, intestine, PMPA, TNFalpha inhibitors

What were your findings with respect to alternative methodologies?

There are no known alternatives to the procedures used in this study.

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?

At the end of the treatment period and/or animals with SAIDS will be euthanised

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 macacques	deep ketamine anesthesia followed by barbiturate overdose	Sodium pentobarbital	60 mg/kg	I.V.

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

--

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.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
--	------------------------------	----------------------

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.

_____ <i>Campus Veterinarian</i>	_____ <i>Date</i>
-------------------------------------	----------------------

09/10/03

Response to pre review questions from
Questions from.

Date: Tue, 09 Sep 2003 15:40:14 -0700

To

From:

>

Subject: pre review questions protocol 10829

Hi,

I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10829. I have attached a copy of the protocol for ease of making revisions.

For this protocol to be considered on the committee agenda of Sept 25th, please forward your revised protocol to me on or before noon, Sept 16th.

Thank you in advance,

Dear ;

We have answered all the questions and concerns that posed in your e-mail note to .

Protocol 10829 ()

1. Section c was very brief and needs to be expanded to include the following information:

a. How are the animals restrained for the various procedures (injections, weighing, sampling, etc)? Are the animals fasted and sedated for any of the procedures?

Animals will be restrained, using ketamine or Telazol as a sedative, prior to any endoscopy procedures or bloods draws, and a combination of medetomidine (30 ug/Kg) and atipamezole (0.15 mg/Kg) will be used to sedate animals prior to lymph node biopsies. Ketoprophen is given as an analgesic (2mg/Kg/day/ for 3 days) after the lymph node biopsies. The animals will be fasted over night prior to the endoscopy procedures or fasted the morning of LN biopsies and bloods draws. Antibiotics are given after jejunal biopsy samples are taken: enrofloxacin (5mg/Kg/day for 10 days) and metronidazol (50mg/Kg orally/day for 10 days). They will not be fasted prior to PMPA and RDP-58 administration. RDP-58 administration will be followed with a special food treat (approved enrichment items and/or pitted dates or apricot granola bars).

b. Expand and explain how you collect the intestinal lavage samples.

At this time we are not considering including intestinal lavages in our protocol and all references to them have been removed.

c. In section d, you provided additional information on RDP58. Please expand to explain more about each of the procedures in section c. Please break the information into key paragraphs for ease of finding the information.

This has been done. Please let us know if you need additional information.

d. Describe the biopsy procedure and include whether any post op analgesics will be included.

Jejunal pinch biopsies will consist of 12 small (20-25 mg each) tissue pieces from which 4 to 6 million cells can be isolated. Briefly: animals, after overnight fasting, will be sedated using Ketamine and/or Telazol. A pediatric endoscope will be utilized to perform the endoscopy procedure. The endoscopy tube is inserted into the mouth and subsequently passed into the esophagus, the stomach and the past the pyloric sphincter and into the upper small intestine. Each pinch biopsy sample is take by a small instrument that threads through the endoscopy tube. Each biopsy sample will be placed into cold buffer (provided in advance from the laboratory). Antibiotics are given after jejunal biopsy samples are taken: enrofloxacin (5mg/Kg/day for 10 days) and metronidazol (50mg/Kg orally/day for 10 days).

There is the risk of intestinal perforation with jejunal biopsies. When a perforation occurs, a jejunal resection will be performed. Briefly: Animals will be anesthetized with Telazol. A ventral midline abdominal incision will be made. The jejunum would be exteriorized and clamped to preserve the vascularity with Doyen forceps. An approximate 3-5 cm section of jejunum would be removed and an anastomosis would be performed using absorbable suture. The abdomen would be lavaged and then closed routinely in 3 layers. Antibiotics are given afterwards: enrofloxacin (5mg/Kg/day for 10 days) and metronidazol (50mg/Kg orally/day for 10 days).

e.

2. In section g, you have listed agents that are not mentioned in section c. Please expand section c to include the use of these agents if they are to be used - describing under what circumstances they are used.

-Buprenorphine is listed in section g because there was a shortage of Telazol at the time and this was cited as a substitute by the veterinarians.

-Antibiotics are given after jejunal biopsy samples are taken:

enrofloxacin (5mg/Kg/day for 10 days) and

metronidazol (50mg/Kg orally/day for 10 days).

-A combination of medetomidine (30 ug/Kg) and atipamezole (0.15 mg/Kg) will be used to sedate animals prior to lymph node biopsies.

-Ketoprofen is given as an analgesic (2mg/Kg/day/ for 3 days) after the lymph node biopsies.

-Oxymorphone as an analgesic will not be used and has been removed from section g.

3. There is no mention of PMPA until section i. Will you also administer PMPA to these animals? If so, please expand to explain the progression of the study.

Yes. We will be administering PMPA to these animals. This was approved in an amendment to this protocol on January 2, 2002. The addition of PMPA to this protocol has been noted in sections a, c, d and e, in bold print and underlined.

1. The date of your literature search box was left blank in section j. Please complete.

This has been done. Thank you.

I hope this helps. Please let us know if there are more questions or concerns. We will get right back to you. All changes have been put in bold font (and underlined when pertaining to PMPA administration).

Thanks so much;

09/24/03

Questions from .

Hi ,

I have received the following committee questions regarding protocol 10829. Please forward the response with the questions to: campusvet@ucdavis.edu.

Thanks in advance,

Protocol 10829 ()

4. The numbers described in section C, D and E do not seem to match up. Please clarify.

This is entirely my error. Thank you for bringing this to my attention. I have corrected the errors. Several sentences were repeated and they have been deleted. All corrections and additional have been underlined. Thank you for your patience.

5. The numbers justification needs a little more substance than " ..to obtain statistically relevant data". Please provide the statistical program you might use that provides the relevant data.

We have experienced significant variation within groups of animals of 5 or less. Because the animal to animal variation has been significant, and because we are designing an experiment where the variation between groups may be small, we will need to have a minimum of 6 animals in each group. We are planning to evaluate statistical significance by using Student's *t* test. Recent publications have indicated that 6 is a minimal number of animals in a group for rhesus macaque studies:

2. Monceaux V, Estaquier J, Fevrier M, Cumont MC, Riviere Y, Aubertin AM, Ameisen JC, Hurtrel B. Extensive apoptosis in lymphoid organs during primary SIV infection predicts rapid progression towards AIDS. *AIDS*. 2003 Jul 25;17(11):1585-96.
3. Vingert BC, Le Grand R, Venet A. Heterogeneity of the simian immunodeficiency virus (SIV) specific CD8(+) T-cell response in mucosal tissues during SIV primary infection. *Microbes Infect*. 2003 Jul;5(9):757-67.
4. Zhao J, Pinczewski J, Gomez-Roman VR, Venzon D, Kalyanaraman VS, Markham PD, Aldrich K, Moake M, Montefiori DC, Lou Y, Pavlakis GN, Robert-Guroff M. Improved protection of rhesus macaques against intrarectal simian immunodeficiency virus SIV(mac251) challenge by a replication-competent Ad5hr-SIVenv/rev and Ad5hr-SIVgag recombinant priming/gp120 boosting regimen. *J Virol*. 2003 Aug;77(15):8354-65.
4. Amedee AM, Lacour N, Ratterree M. Mother-to-infant transmission of SIV via breast-feeding in rhesus macaques. *J Med Primatol*. 2003 Aug;32(4-5):187-93.

More Questions received from yesterday:

I have received the following additional questions for protocol 10829 (). Please send the responses with the questions to: campusvet@ucdavis.edu on or before noon tomorrow so I can forward them to committee members before I leave on vacation.

Thanks in advance,

Protocol 10829 ()

6. Page 1 - special husbandry, typo SIVmac251, not SICmac251 (note: made the typo change on the database) **I have made the correction. Thank you.**

2. Page 2 - Is project funded? **Yes**

7. b. - prolonged restraint (8+) hrs checked - is this correct? **No.** Please explain for how long and why. **That has been removed.**

4. In section c, you made reference to names. We have been asked by the committee to remove reference to names within the body of the protocol. (note: will delete said references in the database version). **I do not see this, other than naming a kind of forcep. But thanks for pointing this out to us.**

5. Section j. literature search -- does not appear to include TNF or TNF inhibitor. Should FTC be included in the search? It does not appear in the protocol. Tenofovir (name for PMPA) also not included in the search. Please clarify. **This TNF inhibitor is novel and we are working with the manufacturer's of this compound. We have definitely run a literature search on this compound in conjunction with SIV, PMPA and FTC. However, we omitted TNFalpha from the list in this section. We have corrected this error. FTC was listed in error. This has been corrected.**