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: These animals will be housed in infectious housing after being infected with SIVmac251.
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.

** 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 TCID 50) 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, 20mg/Kg/day subcutaneously 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 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). 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.

<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>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 .</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>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 .</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>6 animals with no viral infection. sugar water for 20 weeks. Perform jejunal and lymph node biopsy, blood collection, D-xylose absorption .</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>6 animals with SIVmac251 infection only for 34 weeks, jejunal and lymph node biopsy, blood collection, D-xylose absorption .</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>
6 uninfected animals will receive PMPA for 20 weeks, jejunal and lymph node biopsy, blood collection, D-xylose absorption.

<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; <strong>Examples:</strong> domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skilful 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; <strong>Examples:</strong> 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; <strong>Examples:</strong> 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; <strong>Examples:</strong> 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?

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.

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

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 in 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 [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.

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?

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>Current Contents</td>
<td>Sept 1995-2003</td>
<td>AIDS, SIV infection, intestine, PMPA, TNFalpha inhibitors</td>
</tr>
<tr>
<td>Pub Med</td>
<td>Sept 1995-2003</td>
<td>AIDS, SIV infection, intestine, PMPA, TNFalpha inhibitors</td>
</tr>
</tbody>
</table>

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 [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?
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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhesus macaques</td>
<td>deep ketamine anesthesia followed by barbiturate overdose</td>
<td>Sodium pentobarbital</td>
<td>60 mg/kg</td>
<td>I.V.</td>
</tr>
</tbody>
</table>

m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project?
**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/](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/](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/). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

__________________________
Principal Investigator

__________________________
Rank / Title

__________________________
Date

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.

__________________________
Campus Veterinarian

__________________________
Date
ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL #_10829__
EXPIRES: ________

RUA#: ________  BUA#: 0400  CCA#: ________

Identity of Hazard: SIV

Investigator Last Name: __________________________  First Name: __________________________
Email: __________________________  Phone: __________________________

Provide a short description of the agent:
SIV (simian immunodeficiency virus) is a blood born lentivirus that cause fatal immunodeficiency (AIDS) in rhesus macaques. It is genetically similar to HIV. SIV can infect humans but it is unknown whether it can cause disease.

This agent / material is hazardous for: [ ] Humans only  [ ] Animals only  [x ] Humans and Animals

For which Animal Species? [x ] Mammals  [ ] Fish  [ ] Birds  [ ] Invertebrates

The agent can be spread by: [x ] Blood  [ ] Feces/urine  [ ] Saliva/nasal droplets  [ ] Does not leave animal  [ ] Mucosal contact (eye/ mouth/ nose/ genital)

Describe any human health risk associated with this agent:
SIV can infect humans; thus, it could possibly cause fatal AIDS-like disease in humans. SIV-infected humans have generated infectious virus and antibodies to SIV. There have been no reports of disease seen in SIV-infected humans.

The precautions checked below apply to this experiment:
[x ] The researcher or his/her technicians are responsible for the feeding and care of these animals.
[x ] 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:

[x ] Cages must be autoclaved before cleaning.
[x ] Label cages and remove label after decontamination.
[x ] Animal carcasses must be labeled and disposed of as follows:
[ ] Incineration  [x ] Biohazardous Waste Container
[ ] Bag and Autoclave  [ ] EH&S will pick-up (2-1493).

[x ] 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:
[x ] 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
[x ] NIOSH Certified Dust Mask  [ ] Disinfectant footbath
[x ] Eye Protection/Face Shield  [ ]
[ ] Fitted Respirator Type: [x ] Other: Describe: disposable gown/coveralls

[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.
[ ] 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:
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 medetomadine (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.
- Ketoprophen 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:

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 hand 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.**