

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

EH&S USE ONLY

PROTOCOL: 10357
EXPIRES:

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

Species (common names):	Number:	Source:
rhesus	24	CNPRC

Project Title	CpGs as adjuvants in therapeutic HIV vaccines: non-human primate studies		
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Overnight housing location::	CNPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator (If investigator maintained, attach husbandry SOP's.)		

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

Animals will be infected with SIV, put on anti-retroviral therapy (FTC + PMPA) and immunized every two months for six months with either inactivated SIV + CpG ODN or CpG ODN alone. Samples (blood, lymph node biopsies) will be obtained to assess immune responses. Two months after the last immunization, animals will be taken off anti-retroviral therapy and monitored to determine if the immunizations improve the clinical course of infection.

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.

none

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

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

Hazardous Materials (only if in the animal room):

Infectious Agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	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:	NIH, NIAID	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Previous protocol number (if any):	8780

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 pctillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

This project involves immunizing SIV infected, ART (anti-retroviral therapy) treated, rhesus monkeys with a whole-inactivated SIV vaccine in CpG (cytosine and guanine linked by a phosphate, which mimics bacterial DNA) ODN (oligodeoxynucleotide) adjuvant. The ART therapy will consist of PMPA {9-[2-(R)-(phosphonomethoxypropyl] adendine} and FTC {cis(-) amino - 5-flouro-1-[2R,5S-(hydroxymethyl)-1,3 oxathiolan-5-y]-2(1H)-pyrimidinone}. PMPA and FTC block SIV/HIV replication by inhibiting intracellular enzymes the virus needs to replicate. We hypothesize immunizing animals while on ART will generate SIV specific immune responses and boost innate anti-viral defenses. Additionally, the project is designed to determine if therapeutic vaccination during ART with inactivated SIV in CpG ODN adjuvant can provide sustained control of viral replication after ART is discontinued.

b) Procedures employed in this project:

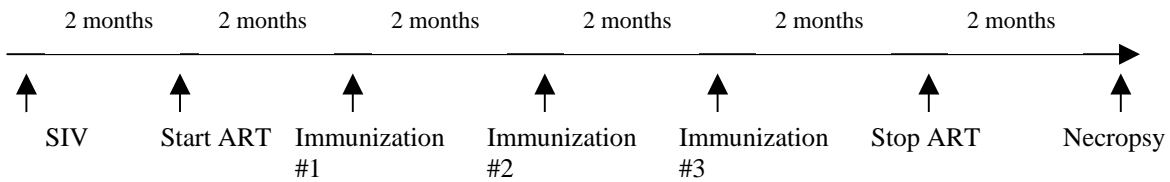
Please check the appropriate boxes if any of these procedures will be employed in your project:

- | | | |
|---|---|---|
| <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 type="checkbox"/> |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

Immunization timeline:



Study outline: Groups A-C

6 months prior to infection	Monthly baseline bleeds
Infection w/SIVmac 251 (dav 0)	10 ml bleed
Post-infection (ni)	2 months of weekly bleeds
ART initiated (2 months pi)	LN biopsy on day initiated 2 months of bleeds every other week, toxicity monitoring
Immunization phase(see above) (2 months after ART initiated)	Blood drawn on the day of, and 1,3,10,14, 28, and 52 days after each immunization. ART tx continued, toxicity monitoring continued. LN biopsy within 7 days of last immunization.
ART continued for 2 months after the last immunization	Blood drawn as stated above. Toxicity monitoring continued.
Animals taken off ART two months after the last immunization.	LN biopsy taken within 7 days after ART stopped. Blood collected the day ART stops.
Monitor for two months after ART stopped	Blood collected every other week for two months
Two months after ART stopped	Animals euthanized and necropsied, lymphoid tissues collected

Note: Group D will have monthly bleeds for six months prior to inoculation, then weekly bleeds for 2 months to monitor infection, and then bleeds every other week starting the day ART is initiated.

GROUP A- Immunization of macaques on ART with inactivated SIV in CpG ODN adjuvant will prevent viral rebound after cessation of ART.

Male juvenile rhesus macaques will be bled monthly for 6 months prior to SIV infection (20-30 ml volume, not to exceed 12 ml/kg/month). This blood will be used for in-vitro assays prior to the start of the study to determine baseline values, and the ability to respond to CpG ODN.

After six months, animals will be infected intravenously with 1 ml (10^5 TCID₅₀) SIVmac251. Blood (5-10 mls, not to exceed 12ml/kg/month) will be collected weekly to monitor the course of infection.

Two months post-infection, animals will be started on ART. This therapy consists of a once daily subcutaneous dose of 30 mg/kg PMPA and 50 mg/kg FTC. This treatment has already been shown to reduce plasma viremia to undetectable levels in chronically infected rhesus macaques. CBCs, chemistry panels and urinalysis will be performed every other week to monitor for potential toxicity. On the day ART is initiated, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution). Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected every two weeks to monitor viral load for two months.

After two months of ART, the animals will be immunized with a combination of 20 ug SIV p27 capsid protein and 500 ug CpG ODN 10103 in 1 ml sterile saline. Half will be administered intramuscularly and half will be administered intradermally. Monkeys will be immunized three times, once every two months. The animals will remain on ART during the immunization phase of the experiment. Blood (5-10 ml, not to exceed 12 ml/kg/month) will be drawn on the day of immunization, and 1, 3, 10, and 14, 28, and 52 days post-immunization. Within seven days after the third immunization, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the last immunization, animals will be taken off ART. Blood will be collected the day animals are taken off ART, and every other week thereafter (5-10 mls, not to exceed 12 ml/kg/month). Within seven days after the cessation of ART, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the cessation of ART, animals will be euthanized, necropsied and lymphoid tissues will be collected for further analysis.

GROUP B- Immunization of macaques on ART with CpG ODN will prevent viral rebound after cessation of ART.

Male juvenile rhesus macaques will be bled monthly for 6 months prior to SIV infection (20-30 ml volume, not to exceed 12 ml/kg/month). This blood will be used for in-vitro assays prior to the start of the study to determine baseline values, and the ability to respond to CpG ODN.

After six months, animals will be infected intravenously with 1 ml (10^5 TCID₅₀) SIVmac251. Blood (5-10 mls, not to exceed 12ml/kg/month) will be collected weekly to monitor the course of infection.

Two months post-infection, animals will be started on ART. This therapy consists of a once daily subcutaneous dose of 30 mg/kg PMPA and 50 mg/kg FTC. This treatment has already been shown to reduce plasma viremia to undetectable levels in chronically infected rhesus macaques. CBCs, chemistry panels and urinalysis will be performed every other week to monitor for potential toxicity. On the day ART is initiated, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution). Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected every two weeks to monitor viral load for two months.

After two months of ART, the animals will be immunized with 500 ug CpG ODN 10103 in 1 ml sterile saline. Half will be administered intramuscularly and

half will be administered intradermally. Monkeys will be immunized three times, once every two months. The animals will remain on ART during the immunization phase of the experiment. Blood (5-10 ml, not to exceed 12 ml/kg/month) will be drawn on the day of immunization, and 1, 3, 10, and 14, 28, and 52 days post-immunization. Within seven days after the third immunization, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the last immunization, animals will be taken off ART. Blood will be collected the day animals are taken off ART, and every other week thereafter (5-10 mls, not to exceed 12 ml/kg/month). Within seven days after the cessation of ART, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the cessation of ART, animals will be euthanized, necropsied and lymphoid tissues will be collected for further analysis.

GROUP C- Immunization of macaques on ART with inactivated SIV in alum will prevent viral rebound after cessation of ART.

One month prior to SIV infection, blood will be collected to establish baseline cytokine RNA levels. (20-30 mls, not to exceed 12 ml/kg/month). Male juvenile rhesus macaques will be infected intravenously with 1 ml (10^5 TCID₅₀) SIVmac251. Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected weekly to monitor the course of infection.

Two months post-infection, animals will be started on ART. This therapy consists of a once daily subcutaneous dose of 30 mg/kg PMPA and 50 mg/kg FTC. This treatment has already been shown to reduce plasma viremia to undetectable levels in chronically infected rhesus macaques. CBCs, chemistry panels and urinalysis will be performed every other week to monitor for potential toxicity. On the day ART is initiated, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution). Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected every two weeks to monitor viral load for two months.

After two months of ART, the animals will be immunized with 20 ug p27 SIV capsid protein in 1ml alum. Half will be administered intramuscularly and half will be administered intradermally. Monkeys will be immunized three times, once every two months. The animals will remain on ART during the immunization phase of the experiment. Blood (5-10 ml, not to exceed 12 ml/kg/month) will be drawn on the day of immunization, and 1, 3, 10, and 14, 28, and 52 days post-immunization. Within seven days after the third immunization, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the last immunization, animals will be taken off ART. Blood will be collected the day animals are taken off ART, and every other week thereafter (5-10 mls, not to exceed 12 ml/kg/month). Within seven days after the cessation of ART, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).

Two months after the cessation of ART, animals will be euthanized, necropsied and lymphoid tissues will be collected for further analysis.

GROUP D- SIV infected animals given ART treatment followed by treatment interruption will experience viral rebound.

One month prior to SIV infection, blood will be collected to establish baseline cytokine RNA levels (20-30 mls, not to exceed 12 ml/kg/month).

Male juvenile rhesus macaques will be infected intravenously with 1 ml (10^5 TCID₅₀) SIVmac251. Blood (5-10 mls, not to exceed 12ml/kg/month) will be collected weekly to monitor the course of infection.

Two months post-infection, animals will be started on ART. This therapy consists of a once daily subcutaneous dose of 30 mg/kg PMPA and 50 mg/kg FTC. This treatment has already been shown to reduce plasma viremia to undetectable levels in chronically infected rhesus macaques. CBCs, chemistry panels and urinalysis will be performed every other week to monitor for potential toxicity. On the day ART is initiated, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution).. Blood (5-10 mls, not to exceed 12 ml/kg/month) will be collected on the day ART is initiated, and every other week from that date.

After six months, the animals will be taken off ART. Blood will be collected the day animals are taken off ART, and every other week thereafter (5-10 mls, not to exceed 12 ml/kg/month). Within seven days after the cessation of ART, a lymph node biopsy will be obtained (peripheral lymph nodes: axillary or inguinal, approx. 1 gram tissue/biopsy) according to CRPRC Standard Operating Procedure. This includes anesthetizing the animal using midazolam as well as topical analgesia via subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 0.25% solution)..

Two months after the cessation of ART, animals will be euthanized, necropsied and lymphoid tissues will be collected for further 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
A	SIV/ART/CpG ODN+inactivated SIV	6	3
B	SIV/ART/CpG ODN	6	3
C	SIV/ART/inactivated SIV in alum	6	3
D	SIV/ART	6	3

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress 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.
2	Minor stress or pain of short duration 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
3	Moderate to severe distress 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
4	Severe pain near, at or above the pain tolerance threshold 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.

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?

There is no alternative to the use of rhesus macaques for this study. The SIV rhesus macaque model is the most generally accepted model of HIV pathogenesis and pre-clinical vaccine studies.

We have decided on six monkeys per group, which will allow us to determine statistically significant differences between groups (using a student T test).

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:

Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
rhesus	Ketamine HCl	10 mg/kg	IM	Prior to all procedures
rhesus	buprenorphine	0.01-0.03mg/kg	IM	As needed in judgement of CRPRC vets
rhesus	midazolam	10 mg/kg	IM	Prior to LN biopsies
rhesus	ketoprofen	20 mg/kg	IM	For three days after LN biopsy
rhesus	lidocaine	0.01 mg/kg	Sub-q	Prior to LN 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)

Any injection or venipuncture has the potential to cause minor pain or discomfort, but the animals are immobilized for the procedure and should not experience pain.

Toxicity from ART should not be a problem at the proposed dosage and duration, however there is a possibility PMPA treatment will decrease the availability of zinc and phosphorus as well as cause kidney dysfunction. To prevent nutrient loss, animals will be given a daily multivitamin when ART is initiated. In addition, animals will be monitored closely via CBC, chemistry panels and urinalysis. If phosphorus levels drop, nutraphos supplements will be given. If kidney problems arise, the PMPA dosage will be dropped to 10 mg/kg. FTC has been linked to a decrease in liver enzyme production. If animals show signs of liver dysfunction, the FTC dosage will be dropped to 30 mg/kg. In our experience using ART for longer durations, these measures alleviated any problems associated with drug toxicity.

SIV infection of rhesus macaques results in a fatal immunodeficiency and wasting syndrome. The animals will be euthanized before, or when, they experience 3 of the following: weight loss >15% in two weeks or >30% in 3 months; persistent hypothermia <96F even with heat supplementation; leukopenia (total WBC <3,000); lymphopenia (lymphocytes <800); anemia (hemoglobin <10); dehydration >10%; nonresponsive to therapy for opportunistic infections; persistent anorexia (>3 days); animal significantly obtunded. These criteria are based on CRPRC guidelines. In addition, the lymph node biopsies will result in some post-procedure pain.

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.

All possible efforts will be made to minimize animal pain and discomfort. Analgesics have no effect on the proposed studies and they will be administered at the discretion of the CRPRC veterinary staff.

Infected animals will be euthanized prior to or at the time they develop clinical signs of AIDS. The decision to euthanize will be based on the judgement of the CRPRC veterinarians.

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: mwood@ucdavis.edu)

What was the date on which you conducted this search?

10/14/02

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

Database Name	Years Covered	Keywords / Search Strategy
PubMed	1990-present	CpG, SIV, anti-retroviral treatment, STI, therapeutic vaccine
Reference Update	1999-present	CpG, SIV, anti-retroviral treatment, STI, therapeutic vaccine
Current Contents	1990-present	CpG, SIV, anti-retroviral treatment, STI, therapeutic vaccine

What were your findings with respect to alternative methodologies?

CpG oligodinucleotides have been shown to induce immune responses in tissue culture systems, rodent models, and cynomolgous monkeys. Thus far, data has not been published using the rhesus macaque model describing CpGs as an adjuvant to a therapeutic vaccine in SIV infection.

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.

Previously, using control animals from a vaccine study, we conducted a pilot project using CpG ODN obtained from another source. The results were inconclusive but hopeful. The new CpG ODN proposed in this study have proven efficacy in other primate models of allergy and infectious disease. Thus, we are repeating and expanding the study to obtain definitive results.

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

Animals will be euthanized and necropsied at the onset of clinical SAIDS or at the end of the study.

l) **Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
rhesus	IV	pentobarbital	60 mg/kg	IV

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

All animals will be euthanized at the end of the project.

ANIMAL ROOM SAFETY INFORMATION**PROTOCOL #** 10357

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

EXPIRES: _____

RUA#: _____

BUA#: 0447

CCA#: _____

Identity of Hazard:

SIV

Investigator Last Name:

Department:

First Name:

Phone:

Email:

Fax:

Provide a short description of the agent:

SIV is a simian retrovirus. This virus can infect human cells and potentially humans.

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

No human disease related to SIV has ever been described. However, there is a potential for SIV to infect humans.

The precautions checked below apply to this experiment:

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

Personal Protective Equipment Required:

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

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

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

BSL 2 (BSL2+) precautions must be used at all times.