

PROTOCOL FOR ANIMAL USE AND CARE
Email to: campusvet@ucdavis.edu
CNPRC

EH&S USE ONLY	
PROTOCOL:	10580
EXPIRES:	5/22/04

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

Species (common names):	Number:	Source:
Rhesus macaque	48	CNPRC

Project Title Effect of vaginal CpG administration on viral resistance and immunity in monkeys.

Overnight housing location:	<input type="checkbox"/> CRPRC	Day use:	<input type="checkbox"/> CRPRC (animal quarters or workrooms)
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

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 treated with CpG (cytosine and guanine linked by a phosphate)ODN (oligodeoxynucleotide)and challenged with SIV (simian immunodeficiency virus).

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.

--

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

- | Sick Animals | Dead Animals | Pest Control |
|--|--|--|
| <input 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 checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	10216

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

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

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pcstillman@ucdavis.edu) 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.

Currently there are no vaccines available to protect people from HIV transmission. Therefore, developing other methods to protect people from transmission, particularly methods that women can control are important to stop the spread of the disease. One of the most attractive options is development of an intravaginal treatment or microbicide. We hypothesize that vaginal CpG (a mimic of bacterial DNA) administration will enhance both innate non-specific immune responses and anti-viral immune responses which will elicit protection against vaginal challenge with SIV. In this study we aim to characterize the immune responses generated in the genital tract, and determine whether these responses are sufficient to provide protection from virus challenge.

b) Procedures employed in this project:

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

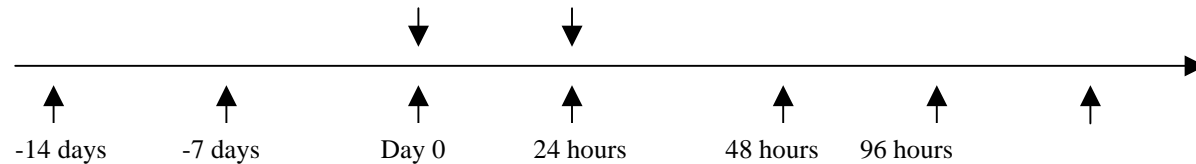
- | | | |
|---|---|---|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery 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.)

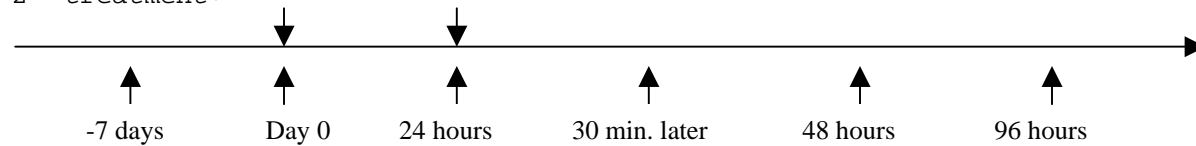
One of the ways the immune system is able to detect microbes is through recognition of unmethylated CpG dinucleotides (CpG motifs) by toll-like receptors. These motifs are common in bacterial DNA, but are under-represented and methylated in vertebrate DNA. Thus, this difference in the DNA allows the immune system to detect foreign microbes and elicit an immune response. Synthetic CpG's have been made in order to take advantage of this ability to activate the immune system. They have been tested for immunogenicity and toxicity in rodents. The results show generation of an immune response with no adverse effects reported. Study timeline below:

Ist treatment:



Two months after the first CpG treatment:

2nd treatment:



↑ = sampling, blood and/or cytobrush and lavage

↓ = CpG ODN administration

*** = SIV challenge 10^5 TCID₅₀ SIV mac239

For all groups: We request mature, cycling female rhesus macaques. For all procedures, animals will be fasted 12 hours prior to immobilization using ketamine (6-10 mg/kg for bleeds and lavages, 20mg/kg for intravaginal CpG administration according to CNPRC standard operating procedures) which will be administered intra-muscularly. Buprenorphine (0.01-0.03 mg/kg) will be administered for post-procedure pain according to the CNPRC veterinary staff.

Group A- Intravaginal administration of 5 mg CpG ODN will generate local and systemic immune responses protecting against SIV challenge.

Blood will be collected (10-20 mls, not to exceed 12 ml/kg/month), a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected 14 days and 7 days prior to the CpG ODN administration. On day 0, 5 mg of CpG-ODN dissolved in 1 ml sterile phosphate buffered saline will be administered intravaginally (by atraumatically inserting a 1 ml syringe). This process will be repeated 24 hours later. Blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals and vaginal lavages (by atraumatically inserting a

pipette into the vagina and washing with PBS) will be collected on Day 0, 12 hours, 24 hours, 48 hours, 96 hours and 7 days after the first CpG administration.

Animals will then be rested for two months, with one blood collection 5 weeks after the first CpG ODN administration. The second CpG treatment will begin two months after the first treatment. At day -7 to the second treatment phase, blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) and lymph node biopsies (peripheral LN's: axillary or inguinal, approx. 1 gram of tissue) will be performed. On day 0 of the second treatment phase, 5 mg of CpG-ODN dissolved in 1 ml sterile phosphate buffered saline will be administered intravaginally (by atraumatically inserting a 1 ml syringe). This process will be repeated 24 hours later. Blood (10-20 mls, not to exceed 12 ml/kg/month) will be collected on Day 0 and at 24 hours. Thirty minutes after the second treatment (24 hours + 30 min.) animals will be challenged with 10^5 TCID₅₀ SIV mac239 in 1 ml. At 48 hours, blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) Blood (10-20 mls, not to exceed 12 ml/kg/month) will be drawn to monitor viral status post-challenge on Day 3, 7, 14, 21, 28, and every month thereafter. Lymph node biopsies (peripheral LN's: axillary or inguinal, approx. 1 gram of tissue) will be performed at 14 days post-challenge and between 3-6 months post-challenge to monitor the dissemination of virus. Animals that become infected will either be culled two to six months post-challenge at which time blood, lymphoid tissues, and reproductive tract tissues will be collected, or will be recycled into another study.

Group B- Intravaginal administration of 10 mg CpG ODN will generate local and systemic immune responses protecting against SIV challenge.

Blood will be collected (10-20 mls, not to exceed 12 ml/kg/month), a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected 14 days and 7 days prior to the CpG ODN administration. On day 0, 10 mg of CpG-ODN dissolved in 1 ml sterile phosphate buffered saline will be administered intravaginally (by atraumatically inserting a 1 ml syringe). This process will be repeated 24 hours later. Blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected on Day 0, 12 hours, 24 hours, 48 hours, 96 hours and 7 days after the first CpG administration. Animals will then be rested for two months, with one blood collection 5 weeks after the first CpG ODN administration. The second CpG treatment will begin two months after the first treatment. At day -7 to the second treatment phase, blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) and lymph node biopsies (peripheral LN's: axillary or inguinal, approx. 1 gram of tissue) will be performed. On day 0 of the second treatment phase, 10 mg of CpG-ODN dissolved in 1 ml sterile phosphate buffered saline will be administered intravaginally (by atraumatically inserting a 1 ml syringe). This process will be repeated 24 hours later. Blood (10-20 mls, not to exceed 12 ml/kg/month) will be collected on Day 0 and at 24 hours. Thirty minutes after the second treatment (24 hours + 30 min.) animals will be challenged with 10^5 TCID₅₀ SIV mac239 in 1 ml. At 48 hours, blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) Blood (10-20 mls, not to exceed 12 ml/kg/month) will be drawn to monitor viral status post-challenge on Day 3, 7, 14, 21, 28, and every month thereafter. Lymph node biopsies (peripheral LN's: axillary or inguinal, approx. 1 gram of tissue) will be performed at 14 days post-challenge and

between 3-6 months post-challenge to monitor the dissemination of virus.

Animals will either be culled two to six months post-challenge at which time blood, lymphoid tissues, and reproductive tract tissues will be collected, or they will be recycled into another study.

Group C- Intravaginal administration of phosphate buffered saline will not generate local and systemic immune responses protecting against SIV challenge.

Blood will be collected (10-20 mls, not to exceed 12 ml/kg/month), a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected 14 days and 7 days prior to the CpG ODN administration. On day 0, 1 ml sterile phosphate buffered saline will be administered intravaginally (by atraumatically inserting a 1 ml syringe). This process will be repeated 24 hours later. Blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected on Day 0, 12 hours, 24 hours, 48 hours, 96 hours and 7 days after the first saline administration. Animals will then be rested for two months, with one blood collection 5 weeks after the first saline administration. The second saline treatment will begin two months after the first treatment. At day -7 to the second treatment phase, blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) and lymph node biopsies (peripheral LN's: axillary or inguinal, approx. 1 gram of tissue) will be performed. On day 0 of the second treatment phase, 1 ml sterile phosphate buffered saline will be administered intravaginally (by atraumatically inserting a 1 ml syringe). This process will be repeated 24 hours later. Blood (10-20 mls, not to exceed 12 ml/kg/month) will be collected on Day 0 and at 24 hours. Thirty minutes after the second treatment (24 hours + 30 min.) animals will be challenged with 10^5 TCID₅₀ SIV mac239 in 1 ml. At 48 hours, blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) Blood (10-20 mls, not to exceed 12 ml/kg/month) will be drawn to monitor viral status post-challenge on Day 3, 7, 14, 21, 28, and every month thereafter. Lymph node biopsies (peripheral LN's: axillary or inguinal, approx. 1 gram of tissue) will be performed at 14 days post-challenge and between 3-6 months post-challenge to monitor the dissemination of virus. Animals will either be culled two to six months post-challenge at which time blood, lymphoid tissues, and reproductive tract tissues will be collected, or they will be recycled into another study.

Group D- SIV control group.

At day -7 to the second treatment phase, blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) and lymph node biopsies (peripheral LN's: axillary or inguinal, approx. 1 gram of tissue) will be performed. Blood (10-20 mls, not to exceed 12 ml/kg/month) will be collected on Day 0. Thirty minutes after the second treatment of Groups A-C (24 hours + 30 min.) animals will be challenged with 10^5 TCID₅₀ SIV mac239 in 1 ml. At 48 hours, blood will be collected (10-20 mls, not to exceed 12 ml/kg/month) a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) Blood (10-20 mls, not to exceed 12 ml/kg/month) will be drawn to monitor viral status post-challenge on Day 3, 7, 14, 21, 28, and every month thereafter. Lymph node biopsies (peripheral LN's: axillary or inguinal, approx. 1 gram of tissue) will be performed at 14 days post-challenge and between 3-6 months post-challenge to monitor the dissemination of virus.

Animals will either be culled two to six months post-challenge at which time

blood, lymphoid tissues, and reproductive tract tissues will be collected, or they will be recycled into another study.

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	5 mg CpG-ODN treatment, SIV challenge	12	3
B	10 mg CpG-ODN treatment, SIV challenge	12	3
C	Phosphate buffered saline, SIV challenge	12	3
D	SIV controls- SIV only	12	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?

The rhesus macaque animal model is the most generally accepted model predication of human immune responses and primates must be used for pre-clinical virus challenge studies of CpG effectiveness. Responses to CpG treatment in our first pilot study were variable, and therefore we request 12 monkeys per group in order to detect statistically significant outcomes

between groups. Fisher's exact test will be used to determine statistical significance between viral load. Anova will be used to determine statistically significant differences in immune responses between protected and unprotected animals.

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	5-10 mg/kg	IM	Before all procedures
rhesus	buprenorphine	0.01-0.03mg/kg	IM	As needed in judgement of CRPRC vets

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

CpG-ODN administration at extreme dosages could possibly result in septic shock. Doses up to 100 ug have been used in the rodent system, and 10 mg intra-tracheally incynomolgous macaque model with no adverse effects reported. A 10 mg dose (the highest dose proposed in this study) in the rhesus macaque is within the same mg/kg dose range, and therefore should not induce adverse effects. As a precaution, animals should not be treated with TNF (Tumor necrosis factor)-alpha during these studies due to the slim possibility of inducing TNF-alpha mediated shock.

SIV infection of rhesus macaques can result in a fatal immunodeficiency and wasting syndrome. The animals will be euthanized when they experience 3 of the following: weight loss >15% in 2 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.

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.

If for some reason an animal goes into shock, it will be treated according to the CRPRC veterinary staff and will not be treated with further CpG-ODN.

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: mwwood@ucdavis.edu)

What was the date on which you conducted this search?

4/15/03

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

Database Name	Years Covered	Keywords / Search Strategy
PubMed	1990-present	CpG motifs, CpG, pathogens, immune response, virus, primates
Reference Update	1999-present	CpG motifs, CpG, pathogens, immune response, virus, primates
Current Contents	1990-present	CpG motifs, CpG, pathogens, immune response, virus, primates

What were your findings with respect to alternative methodologies?

CpG oligodinucleotides have been shown to induce immune responses in tissue culture systems, rodent models, a small number of cynomolgous monkeys, and now a small number of rhesus macaques by group. Intravaginal administration has been found to induce immune responses in the genital tract of some rhesus macaque monkeys. The rhesus macaque animal model is the most generally accepted model predication of human immune responses and primates must be used for pre-clinical studies involving intravaginal administration of CpG-ODN and its effects on local and systemic immune responses. The data we have collected thus far regarding intravaginal administration suggests it could have a protective effect on SIV challenge.

CpG ODN are being tested in various forms to treat allergies and cancer, as well as viral pathogens. They are particularly attractive because they can provide resistance against a broad spectrum of pathogens. Other

adjuvants/agents have been tried with variable success.

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?

Animals will be either A) euthanized at the end of the study or at diagnosis of clinical SAIDS, B) will be recycled onto a different therapeutic study for SIV, or C) protected animals may be recycled and treated again with CpG-ODN

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?

Surplus animals will be: A) will be recycled onto a different therapeutic study for SIV, or B) protected animals may be recycled and treated again with CpG-ODN

ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL # 10580**EXPIRES:** _____

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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 are potentially 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:
- | | |
|--|---|
| <input checked="" type="checkbox"/> Lab Coat/Coveralls | <input checked="" type="checkbox"/> Shoe Covers/Booties |
| <input checked="" type="checkbox"/> Disposable Gloves | <input checked="" type="checkbox"/> Head Cover |
| <input type="checkbox"/> NIOSH Certified Dust Mask | <input type="checkbox"/> Disinfectant footbath |
| <input checked="" type="checkbox"/> Eye Protection/Face Shield | <input type="checkbox"/> |
| <input type="checkbox"/> Fitted Respirator | Type: |
| <input checked="" type="checkbox"/> Other: plastic/disposable gown | 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.

5/5/03

To: , CNPRC

Hi ,

I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10580 for future reference.

I have attached a copy of the protocol for ease of making revisions in response to the questions below.

If you have any questions, please contact me via email or call me at 2-7077.

Thanks in advance,

Protocol 10580 ()

1. In section a, you are asked to provide a brief description of the overall intent of the study and the objectives and significance of the study. You have provided the hypothesis as asked as one of the other parts to the section. Please expand section a to include all parts of the section requested in the instructions.
2. The category listing for the groups was left blank in section d. Since this is SIV work, the category listing should be a 3.
3. In section c, you list ketamine as the agent for immobilizing the animals for the procedures, but in section g, you list telazol and no ketamine. Please clarify which agent you will be using and if both, please list both in sections c as well as g.

Date: Tue, 20 May 2003 14:04:25 -0700

To:

From:

Subject: Fwd: Re: Fwd: Committee questions: Protocol 10580

Date: Tue, 20 May 2003 12:58:08 -0700

To

From:

Subject: Re: Fwd: Committee questions: Protocol 10580

, needs to get an answer ASAP. This goes to meeting this week.

OK, here we go:

Yes, we do anticipate development of clinical SAIDS- we just had another cut and paste issue. Clinical signs are criteria for euthanasia are now included in the attached protocol.

Thanks!

Thanks,

Protocol 10580 ()

1. Under the question on adverse effects, there were no comments about adverse effects seen with SIV infection, but Dr. mentions the possible development of clinical

SAIDs in the section on disposition of animals (k), with the comment that animals will be euthanized with the development of clinical SAIDs. Is the development of any of the signs of clinical SAIDs expected during the time frame of this study? If so, please provide the clinical signs you would expect to see during the study.

2. If you expect clinical signs from SAIDs, shouldn't this information also be included as additional criteria for euthanasia?