

PROTOCOL FOR ANIMAL USE AND CARE*Handwritten forms are not accepted***CNPRC**

EH&S USE ONLY

PROTOCOL # 10215**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 macaque	72	CRPRC

Project Title	Effect of respiratory CpG administration on viral resistance and immunity in monkeys.		
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Overnight housing location::	CRPRC	Day use only :	
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 measles.

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.

Standard infectious housing with quarantine-level precautions for 3 months is required for animals inoculated with wild type measles virus.

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):	Measles
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 type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Previous protocol number (if any):	

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.

The hypothesis of these studies is that administration of CpG's (a mimic of bacterial DNA) will enhance both innate non-specific immune responses and specific anti-viral responses to pathogens.

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. Three CpG's with slight variations in structure (A, B, C) will be used in these experiments. They have been tested for immunogenicity and toxicity in rodents. The results show generation of an immune response with no adverse effects reported. Study design below:

Date	Procedure	Blood draw
Day 0	ODN treatment	10-15 mls
Day 3	No treatment	10-15 mls
Day 7	No treatment	10-15 mls
Day 14	ODN treatment	10-15 mls
Day 28	ODN treatment	10-15 mls
Day 42	ODN treatment	10-15 mls
Day 49	Measles challenge	10-15 mls
Day 7 PC	NP aspirates, BALs	10-15 mls
Day 14 PC	NP aspirates, BALs	10-15 mls
Weekly	No treatment	10-15 mls
Day 60 PC	Necropsy	Maximum

NP= nasopharyngeal

BAL= bronchoalveolar lavage

PC= post-challenge

ODN= oligodeoxynucleotide (with or without CpG's depending on group)

Group A-CpG-A administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 0.3 mg CpG-A (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49

post-treatment to monitor the systemic immune response. Blood (10-15 ml, but not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group B-CpG-B administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 0.3 mg CpG-B (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group C-CpG-C administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 0.3 mg CpG-C (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group D-Control oligonucleotide (containing no CpG motifs) administration will not protect against respiratory infection of Measles.

Male juvenile rhesus macaques will be treated with 0.3 mg control oligonucleotides (dissolved in 1 ml phosphate buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group E-CpG-A administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 1.5 mg CpG-A (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group F-CpG-B administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 1.5 mg CpG-B (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group G-CpG-C administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 1.5 mg CpG-C (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group H-Control oligonucleotide (containing no CpG motifs) administration will not protect against respiratory infection of Measles.

Male juvenile rhesus macaques will be treated with 1.5 mg control

oligonucleotides (dissolved in 1 ml phosphate buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group I-CpG-A administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 4.5 mg CpG-A (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group J-CpG-B administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 4.5 mg CpG-B (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group K-CpG-C administration will protect against respiratory infection of measles.

Male juvenile rhesus macaques will be treated with 4.5 mg CpG-C (dissolved in 1 ml phosphate-buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then

quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

Group L-Control oligonucleotide (containing no CpG motifs) administration will not protect against respiratory infection of Measles.

Male juvenile rhesus macaques will be treated with 4.5 mg control oligonucleotides (dissolved in 1 ml phosphate buffered saline) intranasally (will be introduced drop-wise into the nares) starting on Day 0, then 14, 28, and 42 days after the first treatment. On Day 49, the animals will be challenged intranasally (will be introduced drop-wise into the nares) with 10^5 TCID₅₀ measles virus in 1 ml saline. Nasopharyngeal aspirates (during Ketamine anesthesia, 1 ml sterile saline is instilled in the nose with a soft pediatric catheter and then quickly aspirated) will be performed on all monkeys on days 7 and 14 after measles challenge to monitor viral loads and shedding from the upper respiratory tract. Broncho-alveolar lavages will be performed on days 7 and 14 to monitor viral shedding from the lower respiratory tract. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn on days 3, 7, 14, 28, 42, 49 post-treatment to monitor the systemic immune response. Blood (10-15 ml, not to exceed 12 ml/kg/month) will be drawn weekly post-challenge to monitor measles infection. Animals will be culled two months post-challenge at which time blood, lymphoid tissues, nasal and lower respiratory tract tissues will be collected.

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	Males, intranasal 0.3 mg CpG-A and measles	6	3
B	Males, intranasal 0.3 mg CpG-B and measles	6	3
C	Males, intranasal 0.3 mg CpG-C and measles	6	3
D	Males, intranasal 0.3 mg control and measles	6	3
E	Males, intranasal 1.5 mg CpG-A and measles	6	3
F	Males, intranasal 1.5 mg CpG-B and measles	6	3
G	Males, intranasal 1.5 mg CpG-C and measles	6	3
H	Males, intranasal 1.5 mg control and measles	6	3
I	Males, intranasal 4.5 mg CpG-A and measles	6	3
J	Males, intranasal 4.5 mg CpG-B and measles	6	3
K	Males, intranasal 4.5 mg CpG-C and measles	6	3
L	Males, intranasal 4.5 mg control and measles	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?

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. Six animals for the intranasal challenge with measles is the minimum number per group that will permit us to distinguish statistically significant outcomes (using a student T test) between groups using +/- infection criteria.

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	Telazol	6-8 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 or venipuncture has the potential to cause minor pain or discomfort, but the animals are immobilized for the procedure and should not experience pain.

Rhesus monkeys infected with pathogenic measles virus experience mild discomfort, lethargy and anorexia for several days. Measles causes a skin rash and pneumonia, but the rash does not itch or ulcerate and the pneumonia causes only mild cough and shortness of breath for several days. Post-measles complications are mainly bacterial gastroenteritis.

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.

All clinical signs are noted by staff veterinarians, with discretion to use appropriate palliation and specific anti-microbial therapy.

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:

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search?

6/4/02

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 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, and a small number of cynomolgous monkeys. So far, they have not been tested in the rhesus macaque model, nor have they been tested alone against a pathogenic challenge. They 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.

No.

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

Animals will be euthanized after systemic infection is documented or to collect tissues according to experimental design.

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?

Animals will be euthanized at the end of the study.

Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

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

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

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