**PROTOCOL FOR ANIMAL USE AND CARE**

Handwritten forms are not accepted

**CNPRC**

**Investigator**

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<th>Last Name:</th>
<th>Contact</th>
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<th>After hrs. #:</th>
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**Species (common names):**

<table>
<thead>
<tr>
<th>Rhesus macaque</th>
<th>Number:</th>
<th>Source:</th>
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<tbody>
<tr>
<td></td>
<td>72</td>
<td>CRPRC</td>
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**Project Title**

Effect of respiratory CpG administration on viral resistance and immunity in monkeys.

**Overnight housing location:** CRPRC  
**Day use only:**

Animals will be maintained by:

- [x] Vivarium  
- [ ] 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 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**

- [ ] Call Investigator  
- [x] Clinician to treat  
- [ ] Terminate  
- [ ] Necropsy

**Dead Animals**

- [ ] Call Investigator  
- [ ] Save for Investigator  
- [ ] Bag for disposal  
- [x] Necropsy

**Pest Control**

- [ ] Call Investigator  
- [x] OK to use pesticides  
- [ ] No Pesticides in animal area

**Hazardous Materials (only if in the animal room):**

- [x] Yes  
- [ ] No

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>Agent(s):</th>
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<tbody>
<tr>
<td>Measles</td>
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<th>Radioisotopes?</th>
<th>Agent(s):</th>
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<th>Chemical Carcinogens?</th>
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<tr>
<th>Toxic Chemicals?</th>
<th>Agent(s):</th>
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University of California, Davis  
Printed 10/31/2003  8:16 AM  Page 1
Funding source: NIH, NIAID

Previously approved? [ ] Yes [X] No

Is the project already funded? [ ] Yes [X] No

Previous protocol number (if any):

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

- [ ] Lab Animal Health Clinic (2-0514)
- [] VMTH Large Animal Field Service (2-0292)
- [X] California Primate Research Center (2-0447)
- [ ] 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:

- [ ] Monoclonal Antibody Production **
- [ ] Polyclonal Antibody Production **
- [ ] LD 50 or ID50 studies.
- [X] catheters, blood collection, intubation
- [ ] Prolonged restraint (8 hrs+)
- [X] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [X] Induced illness, intoxication, or disease
- [ ] Survival surgical procedures
- [ ] Death as an endpoint (see i below)
- [ ] Multiple survival surgery
- [X] Trapping, banding or marking wild animals
- [ ] Behavioral modification.
- [ ] Aversive conditioning.

** 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:

<table>
<thead>
<tr>
<th>Date</th>
<th>Procedure</th>
<th>Blood draw</th>
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</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>ODN treatment</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Day 3</td>
<td>No treatment</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Day 7</td>
<td>No treatment</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Day 14</td>
<td>ODN treatment</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Day 28</td>
<td>ODN treatment</td>
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</tr>
<tr>
<td>Day 42</td>
<td>ODN treatment</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Day 49</td>
<td>Measles challenge</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Day 7 PC</td>
<td>NP aspirates, BALs</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Day 14 PC</td>
<td>NP aspirates, BALs</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Weekly</td>
<td>No treatment</td>
<td>10-15 mls</td>
</tr>
<tr>
<td>Day 60 PC</td>
<td>Necropsy</td>
<td>Maximum</td>
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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 $10^5$ TCID$_{50}$ 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 TCID_{50} 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 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 TCID_{50} 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 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 TCID_{50} 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 \(10^5\) TCID\(_{50}\) 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 \(10^5\) TCID\(_{50}\) 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 \(10^5\) TCID\(_{50}\) 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 $10^5$ TCID$_{50}$ 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 $10^5$ TCID$_{50}$ 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 $10^5$ TCID$_{50}$ 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 $10^5$ TCID$_{50}$ 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 $10^5$ TCID$_{50}$ 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Males, intranasal 0.3 mg CpG-A and measles</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>Males, intranasal 0.3 mg CpG-B and measles</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>Males, intranasal 0.3 mg CpG-C and measles</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>Males, intranasal 0.3 mg control and measles</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>Males, intranasal 1.5 mg CpG-A and measles</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>Males, intranasal 1.5 mg CpG-B and measles</td>
<td>6</td>
<td>3</td>
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<td>6</td>
<td>3</td>
</tr>
<tr>
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<td>3</td>
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<td>6</td>
<td>3</td>
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<td>Males, intranasal 4.5 mg CpG-C and measles</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>L</td>
<td>Males, intranasal 4.5 mg control and measles</td>
<td>6</td>
<td>3</td>
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Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| 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.

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

Building:  
Room:  
Who will be the surgeon?

f) Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:

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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>6-8 mg/kg</td>
<td>IM</td>
<td>Before all procedures</td>
</tr>
<tr>
<td>rhesus</td>
<td>buprenorphine</td>
<td>0.01-0.03 mg/kg</td>
<td>IM</td>
<td>As needed in judgement of CRPRC vets</td>
</tr>
</tbody>
</table>

h) Neuromuscular blocking agents can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?
What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:
Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

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 CRPVC 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 [ ] 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.

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Update</td>
<td>1999-present</td>
<td>CpG motifs, CpG, pathogens, immune response, virus, primates</td>
</tr>
<tr>
<td>Current Contents</td>
<td>1990-present</td>
<td>CpG motifs, CpG, pathogens, immune response, virus, primates</td>
</tr>
</tbody>
</table>
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  [X] 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
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<tbody>
<tr>
<td>rhesus</td>
<td>IV</td>
<td>pentobarbital</td>
<td>60 mg/kg</td>
<td>IV</td>
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</table>

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.
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don't need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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Occupational Health Program:
Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://clueless.ucdavis.edu/health/](http://clueless.ucdavis.edu/health/) or read the UC Davis Policy & Procedure Manual 290-25.

Training:
Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at [http://clueless.ucdavis.edu/](http://clueless.ucdavis.edu/).
Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

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

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

__________________________
Principal Investigator

__________________________
Rank / Title

__________________________
Date

** Conditions necessary for Committee Approval:


Final Disposition of this protocol:

_________ Approved
_________ Not Approved
_________ Withdrawn by Investigator

Date of Action: ______/_____/______

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

__________________________
Campus Veterinarian

__________________________
Date

)}
ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL #________

expires: ________

RUA#:

BUA#:

0552a

CCA#:

Identity of Hazard: Measles

Investigator Last Name: ___________________ Department: ___________________

First Name: ___________________ Phone: ___________________

Email: ___________________ Fax: ___________________

Provide a short description of the agent:

Measles infects both monkeys and humans, CRPRC requires vaccination to measles prior to working at the facility. Measles virus is a common respiratory virus that causes a mild illness or no illness in adults.

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

For which Animal Species?

[ X ] Blood (both) [ X ] Feces/urine (measles)

[ X ] Saliva/nasal droplets (both) [ ] Does not leave animal

[ X ] Other: All mucosal secretions can be contaminated. (SIV)

Describe any human health risk associated with this agent:

Measles can be potentially harmful in immuno-compromised adults and pregnant women. Measles can cause abortion in pregnant women.

All of these agents are readily inactivated with 10% bleach and anti-microbial agents for skin care or simple hand washing.

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:

[ X ] Cages must be autoclaved before cleaning.

[ X ] Label cages and remove label after decontamination.

[ X ] Animal carcasses must be labeled and disposed of as follows:

[ X ] Incineration [ ] Biohazardous Waste Container

[ ] Bag and Autoclave [ ] EH&S will pick-up (2-1493).

[ X ] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:

[ ] Incineration [ ] Biohazardous Waste Container

[ ] Bag and Autoclave [ ] EH&S will pick-up (2-1493).

Personal Protective Equipment Required:

[ X ] The following personal protective equipment must be worn/used in the room:

[ X ] Lab Coat/Coveralls [ X ] Shoe Covers/Booties

[ X ] Disposable Gloves [ X ] Head Cover

[ ] NIOSH Certified Dust Mask [ X ] Disinfectant footbath

[ X ] Eye Protection/Face Shield [ ]

[ X ] Fitted Respirator Type:

[ X ] Other: ____________________________________________

Describe: ____________________________________________

[ X ] Personal protective equipment must be removed before leaving the room.

[ X ] Personal protective equipment must be discarded or decontaminated at the end of the project

[ X ] Hands, arms, and face must be thoroughly washed upon leaving the room

[ X ] Full shower, including washing of hair, must be taken upon leaving the room.

[ X ] 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:

For measles, standard quarantine - level precautions are necessary for containment of the virus. Any rash/illness in yourself or a person exposed to you such as a child at home should be reported immediately to a physician. All procedures in the relevant BUA should be followed.