Two rhesus monkeys will be inoculated with HSVgp120 amplicons by the intranasal and intramuscular routes at time 0 and 4 weeks later. Immune responses will be measured prior to immunization and at multiple time points thereafter. The HSV amplicons are replication defective and have no chance of causing disease. They are not live viruses.

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.

N/A

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

Sick Animals
[ X ] Call Investigator  [ X ] Call Investigator  [ X ] Call Investigator
[ ] Clinician to treat  [ ] Save for Investigator  [ ] OK to use pesticides
[ ] Terminate  [ ] Bag for disposal  [ ] No Pesticides in animal area
[ ] Necropsy  [ ] Necropsy

Dead Animals

Pest Control

Hazardous Materials (only if in the animal room):

Infectious Agents?  [ ] Yes  [ X ] No
Agent(s):

Radioisotopes?  [ ] Yes  [ X ] No
Agent(s):

Chemical Carcinogens?  [ ] Yes  [ X ] No
Agent(s):

Toxic Chemicals?  [ ] Yes  [ X ] No
Agent(s):
Is the project already funded? [ ] Yes [X] No

Previously approved? [ ] Yes [X] No

Proposed Funding Source: University Aids Research Program

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.

Infection of humans with members of the herpesvirus family results in long-term, potent immunologic responses, including brisk CD8+ activity. The potency of this response has been exploited to study the use of attenuated herpesviruses, including HSV and varicella zoster virus (VZV) as vaccine vectors. Herpes simplex virus type-1 (HSV-1) has the ability to infect a large number of cells, including dendritic cells (DC) and other potential antigen presenting cells, and its size allows for incorporation of large amounts of foreign DNA. Herpes vectors include both standard gene replacement vectors, as well as highly defective, replication-incompetent plasmid DNAs which contain less than 1% of the viral genome. The latter vectors use a helper virus-free HSV packaging system in which the DNA packaging signals are removed, and the helper virus genome is contained within a bacterial artificial chromosome (bacmid). Viral particles are produced by co-transfecting this bacmid with a plasmid vector that possesses the HSV origin of replication, a viral packaging signal, and the desired gene(s) downstream of a potent promoter. These particles, referred to as HSV amplicons, have multiple, concatameric copies of the desired gene product, and induce both antibody and cellular immune responses in mice, including CD8+ T cell responses.

Based on promising preliminary data in the murine model, we propose to further evaluate the HSV amplicon in Rhesus macaques as a candidate vaccine for HIV-1 immunization. We will carry out vaccination experiments in 2 male macaques using the HIV-1MN/V3-LAI HSVgp120 amplicon. The size of the study is small in order to address four specific questions. First, are the HSV amplicons able to induce both serological and cellular immune responses in rhesus macaques? Second, does the administration of the HSV amplicons by the intranasal route result in detectable mucosal responses to the encoded genes? Third, does prior immunity with a heterologous herpes virus have pronounced effect on the induction of immune responses. Fourth, are these constructs safe when administered to non-human primates?
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.
- Catheters, blood collection, intubation
- Prolonged restraint (8 hrs+)
- Fasting prior to a procedure.
- Non-recovery surgical procedures
- Survival surgical procedures
- Multiple survival surgery
- Behavioral modification.
- Special diets; food or water treatment.
- Food or water restriction
- Special diets; food or water treatment.
- Induced illness, intoxication, or disease
- Survival surgical procedures
- Death as an endpoint (see h below)
- Trapping, banding or marking wild animals
- 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 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.)

Vaccines will be administered IM and intranasally. The animals are anesthetized using ketamine and placed in dorsal recumbancy with the head tilted back. One half ml of vaccine is instilled dropwise into each nostril. The animals are kept in this position for ten minutes and then placed in lateral recumbancy until recovery from anesthesia. During the anesthesia vaccine will also be administered IM in one ml volume in the deltoid muscle.

All the animals will be phlebotomized under Ketamine anesthesia after overnight fasting for blood not to exceed 12 ml/kg body weight per month. A pre-vaccine blood sample will be taken, then post immunization samples will be taken at 0, week 1, week 2, week 3 & week 4 (samples monthly for 6 months). The phlebotomy site is usually the femoral vein, but this is the discretion of the animal health technicians. Blood volume is 6 ml per sample.

The responses to mucosal immunization will be monitored by the use of vaginal washes and oral saliva measurements. Vaginal and oral samples will be taken once a month for six month. In brief, while under the anesthesia, 6 ml of sterile PBS is infused into the vagina and the volume aspirated.

Lymph node biopsies (peripheral lymph nodes: axillary or inguinal, approximately 1 gram of tissue per biopsy) will be taken in month 1, month 3, & month 6 per CRPRC SOP.

Animals will be followed for 6 months. This study is not terminal and animals will be returned to the colony.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; you may add as many rows as you require. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>juvenile</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
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 the species choice was appropriate and the number of animals in the groups above was the minimum number necessary to achieve sound scientific results?

Although it would be preferable to use a large number of primates, this study is only a proof of concept in order to justify further evaluation of more complex and relevant HIV vaccine constructs. The dose of HSV amplicons has been chosen based on the fact that a single laboratory-based run yields approximately $2 \times 10^7$, sufficient for four doses at what we anticipate will be an immunogenic dose in animals of this size. In addition, the ability to scale amplicon manufacture to doses much larger than this for large initial studies (5,000-10,000 doses) in humans using small GMP manufacturing is not practical. Therefore lack of a response at this dose would hamper our enthusiasm for this approach. Second, the availability of macaques limits the number of monkeys to be used. Third, this study is intended to document that there is immunogenicity and safety, and to allow us to design a larger study that would then be funded by the NIH as part of a Program Project to follow.

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

<table>
<thead>
<tr>
<th>Building:</th>
<th>Room:</th>
</tr>
</thead>
<tbody>
<tr>
<td>animal housing</td>
<td>treatment room in animal housing</td>
</tr>
</tbody>
</table>

Who will be the surgeon?  
only minor surgery is proposed, conducted by veterinarian-trained animal health technicians

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

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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhesus</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>phlebotomy, lymph node biopsy</td>
</tr>
<tr>
<td>rhesus</td>
<td>Oxymorphone</td>
<td>0.15</td>
<td>IM</td>
<td>as needed for pain</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)

No adverse effects of amplicon HSVgp120 immunization are anticipated.

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.

Mild analgesic, oxymorphone for post-surgical pain, and antibiotics for some opportunistic infections are administered as needed by the veterinarian.

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? 10/3/00

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>Medline/Pubmed</td>
<td>1996 to 2000</td>
<td>HIV vaccines</td>
</tr>
<tr>
<td>Reference Update</td>
<td>1991 to 2000</td>
<td>Hiv vaccines HSV amplicons</td>
</tr>
</tbody>
</table>
What were your findings with respect to alternative methodologies?

The alternative methodology would be to move directly from mouse to human studies. However, such experiments require the manufacture of GMP material, and prior to committing such resources to even initial larger animal studies, the potential immunogenicity of this approach warrants initial small trials in Rhesus macaques. It is hoped that this small trial will also generate sufficient preliminary data to warrant moving toward a larger funding mechanism (a NIH Program project).

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.

It has never been conducted in macaques

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

The animal will only be euthanized according to other protocols and other studies carried out in the primate center. It is not our intention, to challenge these primates with a matched homologous SHIV. However, if the immune responses are robust, it is likely that we may resubmit a protocol to boost these animals in the future and follow the immune response induction with a SHIV challenge. Those potential experiments are not being planned as part of this protocol.

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

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

There will be no surplus animals.
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/ 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/.
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

____________________   ________
CRPRC Director   Date

Committee Use Only Below

** Conditions necessary for Committee Approval:


Final Disposition of this protocol:

_______ Approved

_______ Not Approved

_______ Withdrawn by Investigator

Date of Action: _____/_____/_____

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

____________________   ________
Campus Veterinarian   Date