

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

EH&amp;S USE ONLY

**PROTOCOL # 9582****EXPIRES: \_\_\_\_\_****Investigator**

Last Name:	
First:	
Middle:	
email:	
Department:	
Phone:	
Fax:	

**Contact**

Last Name:	
First:	
Middle:	
email:	
Department:	
Phone:	
Fax:	

<b>Species (common names):</b>	<b>Number:</b>	<b>Source:</b>
Rhesus Macaques	73	CRPRC, UC Davis

<b>Project Title</b>	Enhanced Safety and Efficacy of AIDS Vaccines by IFN-gamma		
Overnight housing location::	CRPRC	Day use only :	
Animals will be maintained by:	<input 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.

The animals will be vaccinated with recombinant Simian Immunodeficiency Virus (rSIV) and/or Vaccinia Virus and challenged with the pathogenic SIV. The blood, peripheral lymph nodes biopsies, saliva, and vaginal/rectal washes will be taken periodically under proper analgesia or anesthesia.
--

**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.

No special husbandry requirement.
-----------------------------------

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

<b>Sick Animals</b>	<b>Dead Animals</b>	<b>Pest Control</b>
<input checked="" type="checkbox"/> Call Investigator	<input checked="" 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):	Simian Immunodeficiency Virus, Vaccinia Virus
Radioisotopes?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	

Funding source:	NIH	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	8157

**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 long term goal of this project is to develop a safe and efficacious vaccine for human immunodeficiency virus (HIV). We have chosen to use infection of macaques with pathogenic SIVmac as the best model for testing AIDS vaccine safety and efficacy. Several different genetically engineered vaccines for SIV will be tested in rhesus macaques. The effectiveness of the vaccines will be tested by challenge of the animals with virulent SIV or derivatives of SIV. These vaccines include recombinant SIV expressing interferon-gamma or other genes, and vaccinia virus and/or baculovirus recombinant expressing SIV genes (virus like particles, VLPs) and interferon-gamma.

**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 type="checkbox"/> Induced illness, intoxication, or disease |
| <input checked="" type="checkbox"/> LD 50 or ID50 studies.                  | <input checked="" 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.)

Our long term goal is to develop a safe and efficacious vaccine for human immunodeficiency virus (HIV). We have chosen to use infection of macaques with pathogenic SIV<sub>mac</sub> as the best model for testing vaccine safety and efficacy. Work from our laboratory and from many others has shown that virion subunits and live recombinant vectors have provided limited success, and their potential needs to be investigated further as effective vaccines. The only vaccine that has shown efficacy in this model is a live, attenuated SIV, engineered by deleting the nef gene of the virus (SIV $\Delta$ nef), or by deleting nef and two more genetic elements (SIV $\Delta$ 3). The nef gene is one of the accessory genes of SIV, involved in viral pathogenesis, and not required for the replication of virus. A deletion in the nef region attenuates the virus in animals. Although this vaccine provided long-term protection even against high doses of virulent SIV<sub>mac</sub>251, some of its properties preclude its use as a human vaccine: 1) it persists indefinitely in vaccinated macaques; 2) it takes 15 weeks to a year after immunization to establish protective immunity; 3) SIV $\Delta$ 3 has been reported to be pathogenic to neonatal and some adult macaques.

Our work suggests that further attenuation of SIV $\Delta$ nef can be achieved without compromising protection against virulent SIV. This could lead to the generation of a safer attenuated vaccine against HIV. Based on our previous findings that vaccinia virus recombinants expressing interferon-gamma (IFN- $\gamma$ ) are attenuated for immunocompromised nude mice; we replaced the nef gene of SIV with the human IFN- $\gamma$  gene (SIV<sub>H $\gamma$ IFN</sub>). Our preliminary experiments have shown: 1) SIV<sub>H $\gamma$ IFN</sub> is not pathogenic in neonatal macaques, even at an oral dose of 10<sup>5</sup> tissue culture infectious dose 50% (TCID<sub>50</sub>) and intravenous dose of 10<sup>2</sup> TCID<sub>50</sub>; 2) SIV<sub>H $\gamma$ IFN</sub> exhibits a statistically significant lower virus load after immunization of juvenile and neonatal macaques; although all adult macaques became infected after challenge at 6 months postvaccination, yet the ones immunized with SIV<sub>H $\gamma$ IFN</sub> exhibited a statistically lower virus load of SIV<sub>mac</sub>251 than macaques immunized with SIV $\Delta$ nef. Moreover, we have shown complete protection in 25-40% neonatal macaques vaccinated with SIV<sub>H $\gamma$ IFN</sub>.

The studies outlined in this application will permit a more thorough analysis, in larger numbers of animals than in our preliminary feasibility study, of our hypothesis that IFN- $\gamma$  and other lymphokine genes can enhance the safety and protective immunogenicity of an attenuated SIV vaccine. Also, mucosal transmission is the main mode of HIV-1 transmission in human globally. To study the role of mucosal immunity in prevention of infection or disease, the studies are in progress where animals were vaccinated orally with both SIV<sub>H $\gamma$ IFN</sub> and vaccinia virus expressing SIV genes and IFN- $\gamma$ . Alternatively, we have constructed recombinant vaccines based on VLPs and IFN- $\gamma$  expressed in vaccinia virus and/or baculovirus recombinants.

Animals will be vaccinated intravenously, intramuscularly, and/or mucosally with genetically engineered and virulent SIV to assess the immune responses in the blood and at the mucosal surfaces (rectum, vagina, buccal cavity). For our ongoing experiments, monkeys will be vaccinated with recombinant vaccinia virus vaccines either intramuscularly (IM) or orally. If IV challenge with cell free virulent SIV is resisted, other studies will be done to evaluate the efficacy of the vaccine against IV challenge with cell-associated virus and/or mucosal challenge. Animals will be challenged mucosally by applying virulent virus to mucosal membranes. Blood, bone marrow, and lymph node samples will be taken from anesthetized (ketamine) animals by trained CRPRC staff at 1 to 4 week intervals. Samples of blood (5 – 10 ml) will be taken 1,2,4,6,8,12 and thereafter at four week intervals after vaccination or challenge. Animals will be fasted by withholding feed the morning of the day that they are given anesthesia. Blood and tissue samples are usually taken between 9 am and noon. These samples will be used to evaluate the clinical, viral, and immunological status of the animals. If any of the attenuated SIV vaccines appear to be causing disease in neonates, the animals will be euthanized and necropsied. Neonates not exhibiting signs of disease will be kept until the end of the study (three

years). Any adult macaque that is persistently infected with virulent challenge SIV will be kept for approximately three years to evaluate any increase in resistance to terminal disease and will be euthanized once the course of the disease is obvious.

**Sampling schedule (weeks after vaccination or challenge)**

**Blood:**

1(5 ml), 2 (10 ml), 4 (5 ml), 6 (5 ml), 8 (10 ml), 12 (5 ml), 16 (10 ml), 20 (5 ml) Will continue sampling at 4 week intervals, alternating 5 and 10 ml of blood until challenge or euthanasia.

**Lymph nodes:**

2, 4, 8, 16, 24, 32, and at 8 week intervals until challenge or euthanasia.

**To accomplish the specific objectives of the NIH funded project "Enhanced Safety and Efficacy of AIDS Vaccines by IFN- $\gamma$ " (AI36197), and Induction of Mucosal Immunity with Recombinant Vaccines (AI47025 ) the following experiments will be performed within next three years.**

# and Age of Animals*	Vaccination with		Route	SIV Challenge at	
<b><u>Mucosal Immunity (funded by NIH):</u></b>					
5	adult	SIV expressing human IFN- $\gamma$	oral	6-9 months (Vag/Rec)	
5	adult	Recombinant vaccinia virus	oral	6-9 months (Vag/Rec)	
5	adult	Controls	-	Vaginal and/or Rectal	
<b><u>Therapeutic Vaccines (submitted):</u></b>					
5	adult	SIV <sub>mac251</sub>	---	SIV <sub>HyIFN</sub> iv	
5	adult	SIV <sub>mac251</sub>	PMPA	SIV <sub>HyIFN</sub> iv	
5	adult	SIV <sub>mac251</sub>	---	v(SIV <sub>gen</sub> , bSIV <sub>g</sub> iv, im	
5	adult	SIV <sub>mac251</sub>	PMPA	v(SIV <sub>gen</sub> , bSIV <sub>gen</sub> iv, im	
5	adult	SIV <sub>mac251</sub>	PMPA	---	iv (Controls)
5	adult	---	---	SIV <sub>HyIFN</sub> iv (Controls)	
5	adult	SIV <sub>mac251</sub>	---	---	iv (Controls)

Groups will receive PMPA (30 mg/kg of body weight, SQ, daily) for 4 weeks starting on 7 dpi and termination on 35 dpi.

**Oral immunization in neonates (proposed):**

5	neonate	SIV <sub>HyIFN</sub>	oral	6-12 months (iv or mucosal)
5	neonate	SIV <sub>HyIFN</sub>	oral	---
5	neonate	---		(iv or mucosal )

**Determination of AID<sub>50</sub> for challenge virus:**

2	adult	SIV <sub>mac251</sub>	10 <sup>5</sup> TCID <sub>50</sub>	mucosal (intravaginal or rectal)
2	adult	SIV <sub>mac251</sub>	10 <sup>5</sup> TCID <sub>50</sub>	mucosal (intravaginal or rectal)
2	adult	SIV <sub>mac251</sub>	10 <sup>5</sup> TCID <sub>50</sub>	mucosal (intravaginal or rectal)
2	adult	SIV <sub>mac251</sub>	10 <sup>5</sup> TCID <sub>50</sub>	mucosal (intravaginal or rectal)

\* The number of animals per group in the first three experiments are kept to the minimum required for determining valid statistical differences among groups. Five animals per group are the minimum requirement for statistical analysis of the data. We are concerned that the experiment may have to be repeated if the number of animals per group is further reduced. The number of animals used in the mucosal immunity experiment has also been approved in research project funded by NIH.

**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
1	Recombinant SIV and/or vaccinia virus (VV) expressing IFN- $\gamma$ and controls	15 (adult)	2
2	Recombinant SIV and/or expressing IFN- $\gamma$ , PMPA chemotherapy, and controls [see section c) for details]	35 (adult)	2
3	Recombinant SIV expressing IFN- $\gamma$ and controls	15 (neonates)	2
4	SIV challenge virus (for titration in animals)	8 (adults)	2
5	Animals given SIV that progress to AIDS (not all animals will reach this stage) Animals will be euthanized before severe pain or extreme distress occurs due to infection with SIV as per the recommendations of the veterinarians at the primate center.	5 (estimate)	4

#### Categories of invasiveness

Category	Description
1	Little or no discomfort or stress <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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?

SIV is a lentivirus that is genetically related to HIV and causes AIDS-like disease in rhesus macaques. This species is an excellent model for evaluating vaccine and chemotherapeutic approaches for HIV and AIDS.

To determine the statistical differences among the groups, a minimum of 5 animal are required in each group for virus loads, CD4 counts, antibody titers, clinical evaluations etc. among the subgroups. Five animals have been included in each subgroup. These numbers are recommended and approved by the funding agency, National Institute of Health. There is a danger of repeating the experiment due to insufficient data for statistical analysis if the numbers are less than five animals per subgroup.

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

Building:

CRPRC

Room:

CRPRC

Who will be the surgeon?

Veterinarians on duty at CRPRC

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 macaques	Ketamine	10 mg/kg	IM	Once a week before sampling and not more than for 1 hr duration.

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)

The Wyeth strain of vaccinia virus causes a small pock to appear at the site of inoculation. This pock lesion heals within several days. The placement of the vaccination between scapulars restricts the animal's access to the site. The oral administration of VV in chimpanzees and macaques has been cited in literature without any untoward effects.

SIV is fatal to macaques and causes a disease similar to that of AIDS in humans. The clinical signs associated with this disease occur 6 months to 5 years after infection and usually include some, but not all of the following: 1) weight loss; 2) chronic diarrhea unresponsive to treatment; 3) infections unresponsive to antibiotic treatment; 4) inability to maintain body heat or fluids without supplementation; 5) persistent, marked hematologic abnormalities, including lymphopenia, anemia, thrombocytopenia, or neutropenia; and 6) persistent, marked splenomegaly or hepatomegaly. Animals are euthanized when they begin to exhibit one or more of the above signs on the advice of the veterinarians at the primate center.

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.

Vaccination with vaccinia virus recombinants should cause no more discomfort than that of a routine smallpox vaccination and no alleviation of symptoms should be necessary. Since these are recombinant vaccinia viruses and several of the viral genes are inactivated, these vaccines are even more attenuated than the original vaccinia virus vaccine.

If recombinant SIV and VV vaccines fail to provide a complete protection and the animals become infected with virulent SIV, they will be monitored for signs of disease onset (virus load, number of CD4+ T cells, opportunistic infections, immune responses, clinical outcome) and the animals will be euthanized before terminal disease occurs. It is necessary to keep the animals approximately 3 years after challenge to determine if a lower virus load and a favorable clinical outcome (no signs of disease) can be obtained despite the failure of the vaccine to prevent infection with virulent SIV. Whatever routine veterinary treatment needed to alleviate or ameliorate symptoms experienced by SIV-infected macaques will be provided as advised by the veterinarians at CRPRC.

*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.*

1) weight loss greater than 10% in 2 weeks or 30% in 2 months; 2) chronic diarrhea unresponsive to treatment; 3) infections unresponsive to antibiotic treatment; 4) inability to maintain body heat or fluids without supplementation; 5) persistent, marked hematologic abnormalities, including lymphopenia, anemia, thrombocytopenia, or neutropenia; and 6) persistent, marked splenomegaly or hepatomegaly.

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?

Dec 1999 and May 2001

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
Medline (Index Medicus), PUBMED.	Upto 2001	SIV vaccines, vaccinia virus, vaccines, SIV and route of inoculations, SIV and macaques, SIV and Monkeys, SIV pathogenesis, SIV in vitro, IFN- $\gamma$ , Baboons, HIV and Chimpanzee, HIV-1 models, SIV and mucosal immunity, HIV and mucosal immunity, etc

What were your findings with respect to alternative methodologies?

There is no animal model for AIDS caused by HIV-1. Chimpanzees can be infected with HIV-1 but do not get the disease. Baboons have been recently reported to be infected with HIV-2 and get an AIDS-like disease. However, this model has not been used extensively and at present is not as well-defined as SIV in macaques. SIV is a lentivirus that is genetically related to HIV and causes an AIDS-like disease in rhesus macaques. This species is an excellent model for evaluating vaccine approaches for HIV infection and AIDS. Additionally, **in vitro** studies cannot be used to test for an animal's immune response to vaccination. There is no **in vitro** method to study the pathogenesis of virus infection and resistance to challenge with virulent virus.

Has this study been previously conducted?

Yes  No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

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

Animals persistently infected with SIV will be euthanized once the infection is documented and it is determined that this infection will or has led to the development of terminal illness.

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 macaques	anesthetic overdose	pentobarbital	100 mg/kg	IV

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

All SIV-infected animals will be euthanized at the end of the study. Those animals that did not become infected with SIV might be employed in future studies.



