582

**17. Experimental Protocol**

a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated. Be sure to include: all drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary)

Prior to starting the study 20 male animals will be trained to move into a tabletop restraining device, get accustomed to mechanical restraining and electroejaculation procedure (see semen collection below). We will select 4 males that will produce the highest volume of semen, and produce sample on every occasion.

We will establish two cohorts of Rhesus macaques, each containing eight females and four males. 60 days prior to the semen inoculation_We will be selecting four males for each group (for a total of 8 animals) that will supply semen as reliably as possible. The females will be injected i.m. with 30 mg contraceptive DepoProvera to induce vaginal wall thinning thereby increasing the probability of infection. DepoProvera is available at two different concentrations. We would prefer to use the pre-filled syringes, in which it is at 150mg/ml, therefore a single dose is 200 microliters. Otherwise it is also available at 400 mg/ml, which would be a single dose of 75 microliters. At this level, For either dose, DepoProvera is 96-98% effective at preventing pregnancy in humans. The dose level was chosen to prevent pregnancy. In the unlikely event of a pregnancy, the infant will be kept with its mother and will not leave the SIV quarters. The infant will be observed for vertical transmission of SIV. Even if the mother is not infected and the infant tests SIV negative, the infant will remain in SIV quarters to be utilized in other experiments with SIV at a later time. Therefore, the infant will never leave the SIV quarters.

Acute study: Four males per cohort ($A*01^{+}/A*01^{-}$) will be infected intravenously with 100 TCID50 SIVmac239 in less than 1 ml of RPMI 1640, During the acute phase of the infection (week 1-8 post inoculation) animals will be subjected to the electroejaculation procedure twice a week three days apart. Semen will be collected and will be used to inoculate ketamine sedated (10mg/kg) female animals intravaginally. Four female animals will be inoculated twice a week with the inoculations three days apart. Blood draws (7-14 ml) will be collected at the time of semen inoculations from both the infected males and exposed females to look for SIV infection. When the females become infected semen inoculations will be stopped and regular virological and immunological studies will be started. These studies will include weekly blood draws (7-14ml) for the first 8 weeks of infection, followed by monthly blood draws until animals are euthanized. After the females are infected, ketamine will be given i.m. for blood draws.

Chronic study: The same male animals that were infected in the acute phase of the study will be used in the chronic phase of the study. 12 weeks after infection animals will be subjected again to the electroejaculation procedure twice a week three days apart. Semen will be collected and will be used to inoculate ketamine sedated (10mg/kg) uninfected female animals

intravaginally. Four uninfected female animals will be inoculated twice a week with the inoculations three days apart. Blood draws (7-14 ml) will be collected at the time of semen inoculations from both the infected males and exposed females to look for SIV infection. Blood draws will be done under ketamine anesthesia given i.m When the females become infected semen inoculations will be stopped and regular virological and immunological studies will be started. These studies will include weekly blood draws (7-14ml) for the first 8 weeks of infection, followed by biweekly-monthly blood draws (according to the blood collection guidelines of the WPRC) until animals are euthanized.

| Study | # of A*01+ Males | # of A*01+ Females | # of A*01- Males | # of A*01- Females |
|---|---|---|---|---|
| Acute (0-8 weeks) | 4 | 4 | 4 | 4 |
| Chronic (12 weeks on) | | 4 | | 4 |

It is possible that not all female macaques will be infected by the SIV. After at least 3 months of observation following the last inoculation the uninfected animals will be reassigned to other terminal, AIDS related projects. The animals will not leave the WPRC's designated area for SIV research.

**Blood draws**

**The amount of blood obtained from each of these draws will be based on the WPRC blood volume calculations [animal's body weight (kg) x 60 x .10 = maximum volume of blood to be drawn at one time (ml)]. Allowable volumes would be 20% if drawn monthly, 10% if drawn every two weeks, and 5% if drawn weekly. We do not encourage long term weekly blood drawing, although this may be necessary for some experiments. These blood draws are required to allow us to monitor cellular immune responses of the cytotoxic T lymphocytes, helper T lymphocytes, and other immune cells, as well as to obtain antigen presenting cells and B cells for use in experiments. Blood draws may also be necessary to test other parameters such as MHC typing, viral load (if the animals are SIV infected), antibody responses, etc.**

**Blood draws of uninfected animals will be done using a restraint device. In the case where a blood draw is difficult, it may be necessary to sedate the animal as follows: 10 mg/kg ketamine will be used, unless in the opinion of the verterinary staff sufficient anesthesia cannot be obtained with this dose. In this case, 15 mg/kg ketamine will be used, or medetomidine up to 50 ug/kg on top of ketamine at 5 mg/kg, and then reverse with atipamezole up to 250 ug/kg, at the discretion of the veterinarian.**

**Blood draws of SIV infected animals will be done using 10 mg/kg ketamine, unless in the opinion of the verterinary staff sufficient anesthesia cannot be obtained with this dose. In this case, 15 mg/kg ketamine will be used, or medetomidine up to 50 ug/kg on top of ketamine at 5 mg/kg, and then reverse with atipamezole up to 250 ug/kg, at the discretion of the veterinarian.**

At every blood draw we will also collect mucosal secretion samples using the modified wick method described in Kozlowski, P.A. et al 2000. JAIDS Journal of Acquired Immunodeficiency Syndromes 24:297-309. Briefly, a sterile plastic tube applicator containing a premoistened Weck-Cel sponge (triangle shaped, approx. 5x5 mm of size, premoistened with PBS) will be inserted atraumatically in the nasal, vaginal, or rectal cavity. The tip of the sponge will be placed on the mucosal surface and secretion will be allowed to get adsorbed for 5 minutes. After 5 minutes the sponge will be pulled back in the applicator tube which then will be removed from the animal. We will collect samples from all three mucosal surfaces from each animal. This method has been established for human subjects without any apparent adverse effect.

At every blood draw, after the Weck-Cel sample collection we will also collect mucosal secretion samples by lavage from the vaginal or rectal lumen. We will inject <5 ml sterile phosphate buffered saline ph7.4 in the vaginal/rectal cavity with needleless syringe than with the same syringe in place, we will collect about 1-2 ml of the installed fluid. Female macaques will be anesthetized using ketamine (15mg/kg i.m.) by the intramuscular (i.m.) route or ketamine/medetomidine (5mg and 30ug/kg respectively) IM followed by reversal with 150ug/kg atipamezole IM or IV or a more refined anesthetic regimen at the discretion of the veterinarian present. The posterior of the animal will be elevated and 2 – 20 mls (volume depends on the volume of the vaginal vault) of sterile saline or sterile phosphate buffered saline will be used to irrigate the vaginal vault non-traumatically, using

flexible tubing or another flexible device. It is preferable to use the smallest volume possible. It is important to avoid any trauma to the vaginal tissue that could result in contamination of the wash with blood. The procedure will be repeated using another 2 – 20 mls of sterile saline or sterile phosphate buffered saline. Both washes will be collected separately and brought back to the lab for viral analysis. This procedure will be performed at intervals of no less than one per week thoughout the lifetime of the animal to allow a longitudinal analysis of the presence of virus in the vagina as infection progresses. The maximum number of vaginal wash procedures will depend on how long the animal lives. Viral loads go up and down over time, so we will continue to monitor the animal indefinitely, independent of viral load observed. The purpose of this added procedure is to try and ascertain how viral transmission takes place from infected females to male sexual partners, by determining how much virus is present in vaginal secretions. It is known that HIV is transmitted both from male to female and female to male during sexual interactions. However, the amount of virus present in the vagina during different phases of the infection has not been determined. We hypothesize that the virus load in the vagina will rise and fall concomitant with plasma viral loads. This is one hypothesis that we will be testing. We also do not know the kinetics of viral dissemination throughout the animal. We will be infecting these animals intrarectally (i.r.) and know that virus will appear in the vagina, but we don't know if it appears at the same time as the peak viral load in the plasma or if kinetics are delayed. This will also be tested in this experiment. This procedure will not affect the outcome of this study, since this is not a transmission study. However, it will provide data for future transmission studies, enhancing the amount of data which is obtained from a single experiment. In addition the virus obtained from these vaginal washes may be used to infect animals in other studies, making it unnecessary to infect additional female macaques solely to provide vaginal virus for transmission studies.

Macaque semen collection - Selected male animals will be trained to move into a tabletop restraining device, and get accustomed to the mechanical restraining. The duration of training is usually 2-3 weeks. Semen will be collected by penile electro-ejaculation as done routinely at the WPRC (Bavister et al., 1983). Electroejaculation will be performed by aluminum foil electrodes placed on the penis. A slight pulsatile current is required to stimulate ejaculation. The procedure generally takes 10 minutes, but not more than 20 minutes. At least one hour prior to initiating ejaculation, monkeys receive acetaminophen (80 mg PO, 5-10 mg/kg body weight) in marshmallow to prevent any possible discomfort.

b) Do any animals undergo any type of restraint beyond normal housing methods?
**YES**

If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable - available on the web at: www.rarc.wisc.edu

In order to collect semen samples we opted for the physical restraining method. Previously we attempted semen collection on chemically (5-10 mg/kg bodyweight ketamine) restrained animals, and found that ketamine inhibited the reflex of semen ejaculation even at the lowest effective dosage. The length of physical restraint is generally 10 minutes, but not longer than 20 minutes.

Viral challenge and blood draws will be accomplished on animals chemically restrained by intramuscularly administered ketamine hydrochloride at 5mg/kg dose.

c) Are any animals subjected to fluid or food restriction? **NO**

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.)

**YES**

For animals that are infected with immunodeficiency disease inducing viruses, individual housing is the accepted practice. This practice is maintained in order to limit the spread of emerging pathogens from one animal to another.