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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)

We will establish two cohorts of Rhesus macaques, each containing eight females and 16 males. The females will be injected i.m. with 30 mg contraceptive DepoProvera to induce vaginal wall thinning thereby increasing the probability of viral shedding. 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. **The effect of 1 dose of DepoProvera lasts 30 days. A single dose of DepoProvera will be sufficient during the acute phase and another single dose of DepoProvera during the chronic phase will be sufficient.**

Acute study:

Eight females per cohort (either A*01+, B*17+, or A*01-, B*17-) will be infected intravenously with 100 TCID50 SIVmac239 in less than 1 ml of RPMI 1640. During the acute phase of the infection (week 1-4 post inoculation) animals will be **sedated (details of sedation is described at the end of this section)** twice a week, 3-4 days apart for sample collection. We will collect blood (4-8 ml), vaginal fluid by Weck-Cel sponge (**3 samples**), and vaginal lavage by rinsing the vaginal lumen with **two times 3 ml** PBS. Simultaneously we will sedate 8 males and smear their glans of penis at around the opening of the urethra with 200-400ul of the collected vaginal fluid. We will create female-male pairs by exposing the same male with the same females vaginal fluid at every occasion. Prior to exposure to infected vaginal fluid we will collect 4-8 ml blood from the male animals to monitor infection. When the males become infected vaginal fluid transmission 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. All blood draws are performed under sedation.

Chronic study:

The same female animals that were infected in the acute phase of the study will be used in the chronic phase of the study. During the chronic phase of the infection (week 12-17 post inoculation) animals will be sedated twice a week, 3-4 days apart for sample collection. We will collect blood (4-8 ml), vaginal fluid by Weck-Cel sponge (**3 samples**), and vaginal lavage by rinsing the vaginal lumen with **two times 3 ml** PBS. Simultaneously we will sedate 8 males and smear their glans of penis at around the opening of the urethra with 200-400ul of the collected vaginal fluid. We will create female-male pairs by exposing the same male with the same females vaginal fluid at every occasion. Prior to exposure to infected vaginal fluid we will collect 4-8 ml blood from the male animals to monitor infection. When the males become infected vaginal fluid transmission 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. **Progression of SIV induced immunodeficiency disease of all the animals will be monitored by monthly blood draws until animals are euthanized. Parameters to be used for clinical monitoring will be: CBC, Chem-19 panel, CD4 and plasma viral load. All blood draws will be performed under sedation.**

Study	# of A*01+, B*17+ Females	# of A*01+, B*17+ Males	# of A*01- Females	# of A*01- Males
Acute (0-8 weeks)	8	8	8	8
Chronic (12 weeks on)		8		8

Blood draws: Above experimental schedule includes blood volumes that were calculated by using the bodyweight of the smallest animal. Since the majority of the animals included in this study are considerably bigger we may request higher amounts of blood from those animals depending on the goals of actual experiment. The amount of blood obtained from each of these draws will be based on the WPRC blood volume calculations (Bodyweight of animal (kg) x 60 x .05= Maximal volume of blood in mls/week, or bodyweight of animal (kg) x **60** x .20= Maximal volume of blood in mls/month. Blood draws will not exceed 10% of total blood volume at any given time, and no more than 20% of estimated circulating blood volume will be collected in a 30 day period. With each blood draw CBC will be analysed to provide a hematology profile. If anemia is apparent blood draw request will be reduced and iron supplements provided (at the veterinarian's request). Clinically accepted criteria for anemia are lower than normal range values of the RBC, HGB and HCT hematological parameters.

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, premoistened Weck-Cel sponge (triangle shaped, approx. 5x5 mm of size, premoistened with PBS) will be inserted atraumatically in the vaginal 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. This method has been established for human subjects without any apparent adverse effect. We will collect a maximum of 3 samples from the vaginal lumen at each occasion from each animal.

At every blood draw, after the Weck-Cel sample collection we will also collect mucosal secretion samples by lavage from the vaginal lumen. We will inject sterile phosphate buffered saline ph7.4 in the vaginal cavity with needleless syringe than with the same syringe in place, we will collect 5-6 ml of the installed fluid. The posterior of the animal will be elevated and 3 ml (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 1 more time using another 3 mls of sterile saline or sterile phosphate buffered saline. Both washes will be collected separately and brought back to the lab for viral analysis. After the viral transmission study this procedure will be performed at intervals of no less than one per week throughout 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 intravenously 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.

Sedation of animals: macaques will be anesthetized using ketamine (15mg/kg i.m.) or ketamine/medetomidine (5mg and 30ug/kg respectively) i.m. followed by reversal with 150ug/kg atipamezole i.m. or i.v. or a more refined anesthetic regimen at the discretion of the veterinarian present.

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

Viral challenge, blood draws and mucosal secretion sampling will be accomplished on animals chemically restrained by using ketamine (15mg/kg i.m.) or ketamine/medetomidine (5mg and 30ug/kg respectively) i.m. followed by reversal with 150ug/kg atipamezole i.m. or i.v. or a more refined anesthetic regimen at the discretion of the veterinarian present. **The length of restraint is no longer than 45 minutes. The policy of chemically restraining every animal that is exposed to SIV/HIV viruses was introduced to protect personnel, that is involved in animal handling during experimental procedures. These viruses induce immunodeficiency disease, which can result in uncontrolled growth of human pathogens in the infected animals.**

c) Are any animals subjected to fluid or food restriction? **Animals will be deprived of morning food before chemical restraint. The period of deprivation will not be longer than 4 hours.**

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.