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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)

Each animal will be intrarectally inoculated with 3000 Tissue Culture Infectious Doses 50 ($TCID_{50}$) SIVmac239. Blood draws will be taken at weeks 0, 1, and 2 post-inoculation to monitor the development of SIV-specific CTL responses. At 21-days post-infection the animal will be euthanized with sodium pentobarbital and a necropsy will be performed by a trained veterinary pathologist.

Blood draws may be requested before inoculation to assess any pre-existing anti-SIV immunity. All blood draws will adhere to the WPRC guidelines outlined below.

# of Animals	Treatment	Blood draws
5	i.r. SIVmac239	week 0, 1, 2

Inoculation with Live SIV

All animals will be inoculated with live SIV. Challenges will be performed under ketamine anesthesia (up to 15 mg/kg i.m.) or up to 7 mg/kg ketamine HCL i.m. and up to 0.05 mg/kg medetomidine i.m. to be reversed at conclusion of procedure by up to 0.25 mg/kg atipamizole (i.v. or i.m.) or alternative anesthesia in consultation with WPRC veterinarian. Intrarectal challenges will be performed by delivering 3000 TCID50 SIV to the rectal mucosa using a ten cm long feeding tube or tuberculin (Tb) syringe. The actual volume of the virus inoculum varies, depending on the virus titer of the stock. Usually it is <1 ml. Intrarectal challenges will be performed to investigate the protective capacity of a potential vaccine against contact with the virus through mucosal surfaces, as this is the most common transmission route during sexual intercourse in humans.

Blood Draws

The amount of blood obtained from each blood draw 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, as described in SOP 4.01. 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 veterinary staff sufficient anesthesia cannot be obtained with this dose. In

this case, 15 mg/kg ketamine will be used, or medetomidine up to 0.05 mg/kg on top of ketamine at 5 mg/kg, and then reverse with atipamezole up to 0.25 mg/kg, at the discretion of the veterinarian.

Blood draws of SIV infected animals will be done using 10 mg/kg ketamine IM , unless in the opinion of the veterinary staff sufficient anesthesia cannot be obtained with this dose. In this case, 15 mg/kg ketamine IM will be used, or medetomidine up to 0.05 mg/kg IM on top of ketamine at 5 mg/kg IM, and then reverse with atipamezole up to 0.25 mg/kg IM, at the discretion of the veterinarian.

b) Do any animals undergo any type of restraint beyond normal housing methods? **NO** 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

Animals are chemically restrained for many of the procedures, as described in question 17.

c) Are any animals subjected to fluid or food restriction? **YES** If YES, discuss level of restriction, expected consequences, and justification for such restrictions.

Animals will only be food restricted (fasted), **for no more than 20 hours**, the night before the infection or a blood draw, but not for any other reason.

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

If YES, indicated nonstandard husbandry required and justification for this practice.

Animals are individually housed. Please refer to question 35a justification.