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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)

Isolation of Live SIV-derived Virus Population

An SIV infected index animal will be chosen based on similarity to group 1 and distinctiveness from group 2 animals. We will isolate blood from at or near this animal's time of death. From this blood we will extract virus-laden plasma and peripheral blood mononuclear cells (PBMC) using established Ficoll separation methods. If the animal has succumbed to disease, we will process all of the available blood. If the animal is not yet progressing to clinical AIDS then we will request the maximum allowable blood draw using the formula given below.

Challenge with Live SIV

Macaques will be challenged with live SIV. Challenges will be performed under ketamine anesthesia (15mg/kg i.m.) by the intravenous (i.v.) 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. Intravenous (i.v.) challenges will be performed by delivering 2 mL of plasma and 5 x 10^6 PBMC in 2 mLs of phosphate buffered saline into the animal. This dose will be injected slowly and after infection the animal will be returned to its cage. If this inoculation proves insufficient to infect the animal, the viral concentration will be amplified by growth on rhesus PBMC stimulated by phytohemagluttanin. Viruses amplified in this manner routinely achieve a concentration of several thousand TCID50 (Tissue Culture 50% Infectious Doses). If this is used for infection, we will administer 40 TCID50 i.v. in 1 mL, which is known to consistently infect animals. One TCID50 is the dose required to infect 50% of cells in tissue culture. SIV challenges will be performed in the SIV isolation quarters at the Primate Center, where animals will remain until euthanized (see above).

Lymph Node Biopsies

In addition to blood draws, we will perform lymph node biopsies from SIV infected macaques to assess the induction of immune responses in the lymph nodes. The monkey will be anesthetized with ketamine hydrochloride at no more than 15mg/kg 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 fur around the inguinal or axillary lymph node sites will be shaved and the region cleaned with a surgical scrub. A shallow skin incision is used to reveal the lymph node and the tissue is removed with forceps. Local bleeding is stopped by applying pressure to the site or by using an absorbable suture, if necessary. Skin closure is achieved by absorbable subcuticular sutures or by superficial placement of sutures. Animals are monitored daily for 10 days or until the wound is healed. Sutures will be removed after 7 days. Topical antibiotic cream is used as needed as recommended by a veterinarian. No more than two biopsies are collected from any one subject. The interval between biopsies is at least one month and the second biopsy is at a distinct site. We will collect from a single biopsy site in a single procedure.

Biopsies of the vagina and sigmoid colon

In order to determine the cellular composition, function, and antigen specificity of cells derived from mucosal immune compartments within the vagina and sigmoid colon, we will obtain pinch biopsy samples. The monkeys will be anesthetized with ketamine hydrochloride, no more than 15mg/kg, 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. Biopsies will be taken from ten different sites of colon by a fiber-optic flexible pediatric endoscope equipped with biopsy forceps. Size of biopsies will be approximately 2x2x2 mm. We also intend to take pinch biopsies from two different sites of the vagina via a baby Tischler pinch biopsy device, which collects a slightly larger amount of tissue, 3x3x3 mm. Pinch biopsies will be performed four times from three different anatomical sites of an animal before any infection and/or treatment to assess the variability of the samples for individual animals. The interval between biopsies is at least one month and the biopsies will be taken from different sites at each time. Post-operative analgesics: 0.01 - 0.03 mg/kg buprenorphine administered i.m. 0, 12, 24, and 36 hours after the procedure will be provided as recommended by a veterinarian.

Biopsy schedule:

Vagina	Sigmoid Colon	Frequency	Interval between biopsies
2(max)	10(max)	4x(max)	1 month or more

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.

Summary of Animal Treatments:

Group	MHC	# animals	SIV Infection	LN biopsy	Mucosal biopsy
1	Similar	8	+	+	+
2	Disparate	8	+	+	+

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

c) Are any animals subjected to fluid or food restriction? **YES** **NO** If YES, discuss level of restriction, expected consequences, and justification for such restrictions

Animals will only be food restricted (fasted) the night before a procedure, but not for any other reason.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.)
YES **NO** If YES, indicated nonstandard husbandry required and justification for this practice.