**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)

Challenge with Live SIV

Immunized animals will be challenged with live SIV. Challenges will be performed under ketamine anesthesia (15mg/kg i.m.) by the intrarectal (i.r.) route. Intrarectal challenges will be performed by delivering 1000 $TCID_{50}$ SIV to the rectal

mucosa using a ten cm long feeding tube or tuberculin (Tb) syringe. SIV challenges will be performed at the Biotron where animals will remain until euthanized (see above).

Lymph Node Biopsies

In addition to blood draws, we will perform lymph node biopsies from vaccinated and SIV infected macaques to assess the induction of immune responses in the lymph nodes. The monkey will be anesthetized with ketamine hydrochloride (20 mg/kg i.m.). 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 10-14 days if nonabsorbable skin sutures are placed. Topical antibiotic cream is used as needed as per vet. 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. The first biopsy will be the right inguinal and/or axillary lymph node, and the second biopsy will be the left inguinal and/or axillary lymph node. 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 at twice the normal restraint dose (20 mg/kg i.m.) to increase its analgesic properties. Biopsies will be taken from ten different sites of colon by a fiberoptic flexible pediatric gastroscope 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 by 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 the veterinarian.

Biopsy schedule:

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

Bone Marrow Collection:

Animals will be sedated with 5-15 mg/kg ketamine-HCl IM as recommended by the veterinarian. The site to be aspirated (humeral head or iliac crest) will be clipped, a surgical scrub applied and lidocaine will be administered subcutaneously at a 2mg/kg dose, in no more than 0.2 ml volume as a local anesthetic. A heparin-coated 18 gauge spinal needle will be introduced through the skin and will be bored into the bone marrow cavity. The stylet will be removed and no more than 5 ml of bone marrow cells will be aspirated under pressure via a large bore syringe.

**After procedure observation, treatment for complications:**

During the 10 days following sample collection the site of procedure will be observed daily for signs of inflammation, and topical antibiotic ointment will be applied as per the veterinarian recommendation. The bone marrow procedure will only be performed on animals in which testing on already scheduled blood draws indicate that neutralizing antibodies have developed to SIV. Typically the antibody response does not develop until at least 6 weeks post infection, therefore the bone marrow will not be performed until after this time, after the acute phase of SIV infection is completed.

**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 | virus | # animals | Mamu-A*01 | Mamu-B*17 | SIV | LN biopsy | mucosal biopsy | Bone marrow |
|---|---|---|---|---|---|---|---|---|
| A | "pre-escape" | 5 | + | + | + | + | + | + |
| B | "pre-escape" | 5 | - | - | + | + | + | + |
| C | SIVmac239 | 5 | + | + | + | + | + | + |
| D | SIVmac239 | 5 | - | - | + | + | + | + |

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

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.

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 the other.