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2011

You must address questions 17 separately for each species.

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)

Prime with DNA

Genes for Gag, Tat, Rev or Nef are cloned into the [redacted]. 5 mg of plasmid DNA is added to 7.5 mg of [redacted] – a nonionic blocked copolymer adjuvant – [redacted]. Before immunization, the formulation is warmed slowly to room temperature from frozen stock. 1 ml or less of this formulation will then be injected i.m. We will use one limb for each different DNA construct. This is because we, and others, have observed that if an immunodominant epitope (such as CM9 in Gag) is included in the vaccination with other epitopes, the immunodominant epitope suppresses the response to the other epitopes. Therefore, we will use separate limbs for each DNA construct, since in this manner the immune response develops in separate draining lymph nodes, which should avoid this immunosuppressive effect. This prime will be repeated three times at about a 4 week interval.

Boost with Ad5

The adenoviral vector is based on a serotype 5 adenovirus that has been rendered incompetent to replicate by deletion of the E1 and E3 viral genes. Four different viruses will be constructed, encoding each of the four proteins Gag, Tat, Rev or Nef. A total of $10^9 - 10^{11}$ particles of each virus will be injected i.m. in a total volume of 1 ml. We will use one limb for each different virus. This is because we, and others, have observed that if an immunodominant epitope (such as CM9 in Gag) is included in the vaccination with other epitopes, the immunodominant epitope suppresses the response to the other epitopes. Therefore, we will use separate limbs for each adenoviral vector, since in this manner the immune response develops in separate draining lymph nodes, which should avoid this immunosuppressive effect.

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. Intrarectal (i.r.) challenges will be performed by delivering 30 -300 $TCID_{50}$ in a volume of 1 ml to the rectum. This dose will be injected slowly over 1 minute, then the animal's rump kept elevated for an additional 2 minutes before returning the animal to its cate. One TCID50 stand for Tissue Culture Infectious Dose 50, which is the dose required to infect 50% of cells in tissue culture. SIV challenges will be performed in the SIV isolation center at the primate center, where animals will remain until euthanized (see above).

Vaginal Washes

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.

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 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. 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 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 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	SIV Genes	MHC	# animals	Low dose SIV	LN biopsy	mucosal biopsy
3	Gag, tat, rev, nef	Mamu A*01	10	+	+	+
4	Gag, tat, rev, nef	Mamu A*01-	5	+	+	+
5	Vector only	Mamu A*01	10	+	+	+
6	Vector only	Mamu A*01-	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

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.