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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)

Experimental Schedule and Explanation for Required Pilot Study

Part one: To test *in vivo* the infectivity of a newly produced $SIV_{mac239nef\ open}$ virus stock. One animal will be inoculated once intrarectally with the 3000 TCID50 SIV stock. Past results have shown this amount of virus (titer determined *in vitro* by co-cultivation with susceptible CEMx174 cells) to successfully infect the majority of animals exposed within an experiment. If tests demonstrate that this animal has successfully been SIV infected, then the second portion of the pilot study will be started. If the animal is not successfully infected, further *in vitro* tests to determine if the new virus stock contains infective particles will have to be performed before proceeding to the second phase.

Part two: To test for successful infection of four animals by intrarectal delivery of 30 (two animals) or 300 (two animals) TCID50 SIV once weekly for a period of up to 10 weeks. The 30 TCID50 SIV group will be inoculated first, with two weeks between the initial and second virus dose. If both animals are found not to be infected at the end of the initial two week period, they will then be exposed to weekly inoculations for up to ten weeks or until successfully infected. Also, a second group (two animals) will then be started at the 300 TCID50 SIV dose and exposed weekly with virus inoculations for up to ten weeks or until successfully infected. If either or both of the animals in the first group (30 TCID50 SIV) are successfully infected after the initial exposure, the virus dose for the second group of two animals will be reduced to 3 TCID50 SIV (rather than 300 TCID50 SIV).

Blood samples (~6 ml per animal/time) to determine successful infection will be drawn just before each virus inoculation, and on day seven for each group. Periodic mucosal swab samples may also be taken via a non invasive method for defining characteristics of the mucosal membranes after infection. Both virus i.r. inoculations and blood draws will be performed under . The monkeys will be anesthetized using 5-10 mg/kg ketamine HCl and 0.03 mg/kg medetomodine IM to be reversed by 0.15 mg/kg atipamezole IV/IM at procedures end as described below. If at any point tests indicate an animal is successfully infected, further virus inoculations will be stopped. Each infected animal will then be followed over time on a routine basis to further study the pathogenesis and progression to SAIDS induced by this specific virus stock. Blood for CBC, serum chemistry, viral load and lymphocyte counts will be performed at least twice annually. These results will be provided to the veterinary staff to aid in clinical management. When the veterinarian determines that an animal is displaying signs of advanced disease progression, it will be euthanized for procurement of appropriate tissue samples to determine the viral loads and sequence virus particle changes. Uninfected animals (exposed to SIV) will be assigned to other appropriate SIV research projects.

If repetitive i.r. exposures with these smaller quantities of SIV result in a high percentage of infected animals, this method will be used for challenging vaccinated and control animals described in other submitted protocols.

Method for SIV Inoculation

Immunized animals will be challenged with live SIVmac 239nef open. All inoculations will be performed using 5-10 mg/kg ketamine HCl and 0.03 mg/kg medetomodine IM to be reversed by 0.15 mg/kg atipamezole IV/IM at procedures end. Virus (3 to 3,000 TCID50 SIV in ~3-5mls of media) will be delivered onto the rectal mucosa using a ten cm long feeding tube and syringe.

In future research projects, intrarectal challenges will be performed to investigate the protective capacity of potential vaccines against contact with the virus through mucosal surfaces, which is the most common transmission route in humans. If in this pilot study, repetitive i.r. exposures with smaller quantities of SIV result in a high percentage of successfully infected animals, this method will be used for challenging future vaccinated and control animals.

Lymph Node Biopsies

As the disease progresses it may be necessary to perform lymph node biopsies from SIV infected macaques to assess induction of immune responses. The monkey is anesthetized using 5-10 mg/kg ketamine HCl and 0.03 mg/kg medetomodine IM to be reversed by 0.15 mg/kg atipamezole IV/IM at procedures end. The fur around the inguinal lymph node site is 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 then monitored daily for 10 days or until the wound is healed. Sutures are 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 will be collected from any one subject. The interval between biopsies will be at least one month and the second biopsy will be a distinct site. We will collect from a single biopsy site in a single procedure.

Biopsies of sigmoid colon

Also during disease progression it may be necessary to obtain pinch biopsy samples in order to determine the cellular composition and function of mucosal immuncompartments that reside in sigmoid colon. The monkeys will be anesthetized using 5-10 mg/kg ketamine HCl and 0.03 mg/kg medetomodine IM to be reversed by 0.15 mg/kg

atipamezole IV/IM at procedures end. 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. Local bleeding will be stopped by applying pressure to the vessel. The interval between biopsies will be at least one month and the biopsies will be taken from different sites 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:

Sigmoid Colon	Frequency	Interval between biopsies
10(max)	4x(max)	1 month

Blood Draws

The amount of blood obtained from each draw will be based on the WPRC blood volume calculations (Bodyweight of animal (kg) x 60 x .10= Maximal volume of blood to be drawn at one time (in ml). Allowable volumes would be 20%, if drawn monthly; 10% if drawn every two weeks; and 5% if drawn weekly. Routine CBC's (will be analyzed to provide a hematology profile. If anemia is apparent, blood draw requests will be reduced and iron supplements provided (as directed by the veterinarian). These blood draws are required to monitor cellular immune responses (both CTL and T helper responses) and to measure the viral load in SIV-infected animals. If any animal shows an adverse hematology profile, the veterinarian will be consulted before proceeding with additional blood draws. . The monkeys will be anesthetized using 5-10 mg/kg ketamine HCl and 0.03 mg/kg medetomodine IM to be reversed by 0.15 mg/kg atipamezole IV/IM at procedures end.

Mucosal Samples

Mucosal secretion samples will also periodically be collected using the modified wick method described in Kozlowski, P.A. et al 2000. JAIDS Journal of Acquired Immunodeficiency Syndromes 24:297-309. Briefly, a sterile plastic tube applicator containing a premoistened Weck-Cel sponge (triangle shaped, approx. 5x5 mm of size, premoistened with PBS) will be inserted atraumatically in the nasal, oral, vaginal, or rectal cavity. The tip of the sponge will be placed on the mucosal surface and secretion will be collected for 5 minutes. After 5 minutes the sponge will be pulled back in the applicator tube which then will be removed from the animal. We will collect samples from all four mucosal surfaces from each animal (if applicable). This method has been established for human subjects without any apparent adverse effects.

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.

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.