763

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:

High Dose Viral Challenge:

Each animal will be challenged with dilutions of the viral stock of SIVmac239. Animals will be intrarectally inoculated with $10^{-3.5}$, $10^{-4.5}$, $10^{-5.5}$, $10^{-6.5}$ or $10^{-7.5}$ dilutions of the SIVmac239 stock. The actual volume may vary, but usually is less than 1 ml. Challenges will be performed under ketamine anesthesia by intrarectal (i.r.) route. Both virus i.r. inoculations and blood draws will be performed under anesthesia. **The monkeys will be anesthetized using 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.

After 7 days, blood will be drawn and plasma tested for presence and amount of virus, determined by qPCR. Animals will also be assessed for immune responses in the CTL and HTL compartments, as well as for antibodies to SIV proteins at this time to confirm viral load data. In the event that this dose does not infect the animal, as determined by viral load determination and/or immune responses, the animal will be challenged again, possibly with a higher dose. This will be repeated until the animal in infected.

Low Dose Viral Challenge

Animals will be intrarectally innoculated with 30 – 300 TCID50 of SIV stock no more than once weekly for a period of up to 40 challenges or until successfully infected. Challenges will be performed under ketamine anesthesia by intrarectal (i.r.) route. Blood samples (~6 mL per animal per time) will be drawn to determine successful infection before each virus inoculation. Both virus i.r. inoculations and blood draws will be performed under anesthesia. The monkeys will be anesthetized using 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. If at any point tests indicate an animal is successfully infected, further virus inoculations will be stopped. Each infected animal will then enter immunological studies.

Group	# of Animals	Treatments	Blood draws after known infection
1	4	challenge with $10^{-3.5}$ dilution of virus stock.	week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20
2	4	challenge with $10^{-\backslash 4.5}$ dilution of virus stock.	week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20
3	4	challenge with $10^{-5.5}$ dilution of virus stock.	week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20
4	4	challenge with $10^{-6.5}$ dilution of virus stock.	week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20
5	4	challenge with $10^{-7.5}$ dilution of virus stock.	week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20
6	4	challenge with 30 - 300 TCID50 dose up to 40 times. After infection:	week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20

For details on blood draws see below.

Rectal and Vaginal Washes

While animals are under anesthesia for blood draws, we may also collect mucosal secretion samples from the rectum (either gender) and/or vagina (in female animals only). Vaginal wick may or may not be collected. But, if it is collected, it must be collected before vaginal wash collection. 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

For collecting vaginal wick, a premoistened (with HEPES buffer) Weck-Cel sponge (triangle shaped, approx. 5x5 mm of size, premoistened with PBS) will be inserted atraumatically into the vaginal or rectal lumen. The tip of the sponge will be placed on the mucosal surface and the secretion be allowed to be adsorbed for 5 minutes. After 5 minutes, the sponge will be pulled back into the applicator tube and removed from the animal. This method has been established for human subjects without any apparent adverse effect. We will collect up to 3 samples from the vaginal and/or 1 sample from the rectal lumen at each time point from each animal.

For collecting vaginal or rectal wash, a sterile red rubber catheter will be placed vaginally with insertion to full length of vagina, or into the rectal lumen. A syringe containing 3 ml of sterile PBS will be placed on end of catheter and infused into vagina until the fluid level is visualized at vaginal entrance or rectal opening. PBS will be gently flushed in and out 5 times. This is repeated again, a total of ~5-6 mls of fluid will be collected.

After the viral transmission study 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 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. However, it will provide data for future transmission studies, enhancing the amount of data that 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 for the induction of immune responses in the lymph nodes by the vaccine. 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. 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 5 days or until the wound is healed. Sutures will be removed after 7-14 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 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 Sigmoid Colon

In order to determine the cellular composition and function of mucosal immuncompartments that reside in sigmoid colon we **may** obtain **pinch** biopsy samples. **The monkeys will be anesthetized using ketamine (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,** 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 is 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. perioperatively and additional doses as recommended by a WPRC veterinarian. Analgesics (Tylenol 6-10 mg/kg p.o. or Ketoprofen (2-5 mg/kg i.m.)) and broad spectrum antibiotics (*i.e.* cefazolin 20-30 mg/kg i.m. or s.c. twice per day) will also be given to animals if deemed necessary per the veterinarian. This procedure may be combined with a lymph node biopsy, if scientifically reasonable, to minimize anesthetic events for the animal. Animals will have no more than 4 biopsies of the colon and each must be at least one month apart, for a total of 4 anesthetic events due to colon biopsies.**

Biopsy schedule:

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

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.

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