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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 Protocol

Experiment 1: To establish conditions for a SIVmac239 challenge regimen characterized by repeated intravaginal deposition of low infectious virus containing inoculum.
We will inoculate 2 animals with 30,000 TCID50, 2 animals with 3,000 and 2 animals with 300 TCID50 SIVmac239 intravaginally at weekly intervals. We will repeat inoculation until the first positive virus detection in the blood, but not more then ten weeks. The volume of virus inoculum will be **≤ 2.5 ml** at each time. We will assess infection by sampling the peripheral blood before every inoculation (6 ml sample size). Pathogenesis will be monitored by regular immune and molecular biology assays from blood samples: weekly for 1 month after the first positive virus detection, biweekly for the following 2 months, and monthly thereafter. 3 weeks and 12 weeks after the first positive virus detection we will request a large amount of blood to detect emerging immune responses. (In assessing the volume of the large blood draw we will follow the WPRC guidelines detailed below).

Experiment 2: To establish the conditions for effective immune response elicited with Chlamydia trachomatis Serovar E.
Part A./ We will inoculate 2 animals with 10^7 and 2 animals with 10^5 Chlamydia trachomatis Serovar E. The microbe suspension will be deposited onto the cervical os in < 1.5 ml volume. We will perform three separate inoculations 8-12 weeks apart. Infection will be monitored by immune and molecular biology assays from peripheral blood (6 ml sample size) and cervical cytobrush samples biweekly after every Chlamydia inoculation. If animals will not clear infection by two weeks after each inoculation we will treat them with Azithromycin, or other antibiotic as recommended by the veterinarian.

Part B./ To obtain more information on Chlamydia induced immune responses we will inoculate 4 more animals with either 10^7 or 10^5 Chlamydia trachomatis, The microbe suspension will be deposited onto the cervical os in < 1.5 ml volume. The choice of dosage will depend on the results of part A. of the experiment. We will perform three separate inoculations 8-12 weeks apart. Infection will be monitored by immune and molecular biology assays from peripheral blood (6 ml sample size) and cervical cytobrush samples biweekly after every Chlamydia inoculation. If animals will not clear infection by two weeks after each inoculation we will treat them with Azithromycin, or other antibiotic as recommended by the veterinarian.

Experiment 3: To assess the protection rendered by our vaccination protocol against repeated intravaginal SIVmac239 challenge of low inoculation dose.

Control group: We will inoculate eight animals with non-recombinant Chlamydia trachomatis Serovar E, according to an inoculation protocol found in experiment 2 to be the best in eliciting strong immune responses. Infection will be monitored by immune and molecular biology assays from peripheral blood (6 ml sample size) and cervical cytobrush samples biweekly after every Chlamydia inoculation.

Test Group: We will inoculate eight animals intracervically with recombinant Chlamydia trachomatis Serovar E containing tat, rev, nef and gag SIVmac239 genes according to an inoculation protocol found in experiment 2 to be the best in eliciting strong immune responses. Infection will be monitored by immune and molecular biology assays from peripheral blood (6 ml sample size) and cervical cytobrush samples biweekly after every Chlamydia inoculation.

6-8 weeks after the last Chlamydia inoculation we will challenge the animals of both the Control and Test group according our challenge protocol established during experiment 1.
We will assess SIV infection by sampling the peripheral blood before every inoculation (6 ml sample size). Pathogenesis will be monitored by regular immune and molecular biology assays from blood samples: weekly for 1 month after the first positive virus detection, biweekly for the following 2 months, and monthly thereafter. 3 weeks and 12 weeks after the first positive virus detection we will request 12-16 ml blood to detect emerging immune responses.

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.

Lymph Node Samples

In addition to blood draws, we may perform lymph node biopsies from vaccinated and SIV infected macaques to assess for the induction of immune responses in the lymph nodes by the vaccine. Monkeys are anesthetized with 5 mg/kg ketamine hydrochloride + 0.03 mg/kg medetomidine administered IM, followed by **0.15 mg/kg** atipamezole IM or IV.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 **within 10 days** if nonabsorbable skin sutures are placed. Topical antibiotic cream is used as needed as per veterinarian's request. No more than two sampling will be performed on 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 or axillary lymph node, and the second biopsy will be the left inguinal or axillary lymph node. We will collect from a single site in a single procedure.

Chlamydia inoculation

Inoculation will be performed under ketamine anesthesia (15mg/kg I.M.). Cervix will be visualized via a pediatric speculum. Inoculum will be deposited atraumatically onto the cervical os by a steril 2 ml serological pipette.

Cytobrush sampling

We will collect cervical cells from animals with a cytobrush. Animals will be chemically restrained by intramuscularly administered ketamine hydrochloride at 10mg/kg dose. Cervix will be visualized via a pediatric speculum. Cervical cells will be obtained by inserting and gently rotating a cytobrush into the cervical canal.

Challenge with Live SIV

Challenges will be performed under ketamine anesthesia (15mg/kg I.M.). SIVmac239 will be deposited atraumatically into the vaginal vault by a 1 ml tuberculin (Tb) syringe without needle. The amount of viral dose and frequency of viral challenge will be determined in **Experiment 1.**

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

Blood draws will be accomplished on animals chemically restrained by intramuscularly administered ketamine hydrochloride at 10 mg/kg dose. Lymph node removals will take place on animals chemically restrained by 5 mg/kg ketamine hydrochloride + 0.03 mg/kg medetomidine administered IM, followed by 1.5 mg/kg atipamezole IM or IV.

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

Under general anesthesia some animals vomit, so to avoid unnecessary complications on the day of sample collection performed under ketamine restriction morning **food and water may be withheld**. Animals will not be deprived of water longer than 3 hours prior to the 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.

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. Additionally to limit Chlamydia transmission between co-housed animals we will request exemption from pair-housing from the first time of Chlamydia inoculation until the end of the experiment.