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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 TCID<sub>50</sub>, 2 animals with 3,000 and 2 animals with 300 TCID<sub>50</sub> SIVmac239 intravaginally at weekly intervals. We will repeat inoculation until the first positive virus detection in the blood, but not more than 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<sup>7</sup> and 2 animals with 10<sup>5</sup> *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 veterinary 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 veterinary 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.

