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)

An outline of the experimental design was presented earlier (Q. 11a). Following are detailed descriptions of individual procedures that will be used in this project.

1. Blood Drawing: Blood samples will be collected for analysis by venipuncture from either the saphenous or femoral veins. We will want pre-experimental blood draws at the beginning of the experiment for antibody testing, as well as blood samples just prior to sacrifice to determine final plasma SIV viral loads. The amount of blood required at each time point would be approximately 12mls. Total amounts over a period of time would be monitored so as to remain within limits suggested in the SOP 4.12 (Blood volumes guidelines - rhesus) of the Primate Center. Each monkey will be temporarily immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular-with additional small aliquots administered, if animal begins to prematurely recover from anesthesia). The animal will be observed throughout this period and until it has completely recovered from the anesthesia.

2. Virus Inoculation: Virus dilutions at $1x10^5$ $TCID_{50}$ (in ~ 1ml) x 2 inoculations separated by 4 hours (SIVmac251) will be given intravaginally using a soft nasogastric catheter. There is no trauma at the inoculation site. SIV is infectious for human and monkey primary lymphocytes. Each monkey will be temporarily immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular-with additional small aliquots administered, if animal begins to prematurely recover from anesthesia). The animal will be observed throughout this period and until it has completely recovered from the anesthesia.

3. Chlamydia Infection: One ml of 10,000 inclusion forming units (IFU) of C. trachomatis washed over the cervical outer surface of monkeys will infect 100%, assayed by ligase chain reaction on cervical swabs collected 2, 7, 14 days post-inoculation (Sexually Transmitted Diseases 23: 461-464, 1996). World authority on Chlamydia [redacted] agreed to send us the inoculum at this or higher concentration. The stock of Serovar D human endometrial isolate of C. trachomatis is 6 x 10^6 IFU/ml in sucrose-phosphate glutamate buffer. Each monkey will be temporarily immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular-with additional small aliquots administered, if animal begins to prematurely recover from anesthesia). The animal will be observed throughout this period and until it has completely recovered from the anesthesia.

4. Cervical swabs: will be collected during screening of animals, and on 0, 2, 7, 14 days post-inoculation; and will be assayed by ligase chain reaction for the detection of C. trachomatis. Each monkey will be temporarily immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular-with additional small aliquots administered, if animal begins to prematurely recover from anesthesia). The animal will be observed throughout this period and until it has completely recovered from the anesthesia.

5. Anti-inflammatory agent: Topical tacrolimus (FK506) will be applied as the anti-inflammatory agent prior to viral infection as determined in the pilot efficacy study . This agent is well adsorbed and effective in inflammatory conditions of the skin and gut. It is also safe and FDA-approved at a concentration of 0.1%. The 0.1% tacrolimus will be dissolved in polyethylene glycol (MW 400 to 600). This will be a slightly viscous solution injected intra-vaginally in a 1ml volume close to the fornex. We're expecting that any efficacy changes in SIV infection will be due to inhibition of pro inflammatory cytokine expression and T-cell migration and activation (the effect we want to test) (ref. 6). As described above, a preliminary experiment on two animals will be performed to better determine the exact time and applications appropriate for resolving the Chlamydia induced inflammation.

Each monkey will be temporarily immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular-with additional small aliquots administered, if animal begins to prematurely recover from anesthesia). The animal will be observed throughout this period and until it has completely recovered from the anesthesia.

6. Biopsies of the vagina and cervix.: We will perform sample biopsies, presumably only in the preliminary study evaluating the efficacy of the anti-inflammatory agent. The monkey will be immobilized at the front of the cage (by squeeze back mechanism) and anesthetized with an intramuscular injection of ketamine hydrochloride at 20mg/kg. Biopsy samples will be taken from three different sites of the vagina and cervix utilizing a baby Tischler punch biopsy device. The interval between any biopsies will be at least one week with a maximum duration time of three weeks. The biopsies will be taken from different sites each time, and no other biopsy samples will be taken simultaneously.

Each monkey will be temporarily immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular-with additional small aliquots administered, if animal begins to prematurely recover from anesthesia). The animal will be observed throughout this period and until it has completely recovered from the anesthesia. Sample biopsies are considered as minor surgeries with a very small probability of adverse effects. Any subjects of these procedures displaying visible signs of discomfort will be treated with an intramuscular injection of Buprenorphine at 0.01mg/kg once a day for two days.

7. Cytobrush collection of cervical cells: We will also collect cervical cells from these female animals utilizing a cytobrush, which will collect cells from the cervix, allowing analysis of cell populations correlated to inflammation. This procedure will be performed just prior to any biopsy samples taken. The cervix will be visualized by a pediatric speculum, and cervical cells will be obtained by inserting and gently rotating a cytobrush used to obtain samples for human PAP smears.

Each monkey will be temporarily immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular-with additional small aliquots administered, if animal begins to prematurely recover from anesthesia). The animal will be observed throughout this period and until it has completely recovered from the anesthesia. **Cytobrush sample collection from the cervical canal will result in only a slight discomfort** with a very small probability of adverse effects. Any subjects of these procedures displaying visible signs of discomfort will be treated with an intramuscular injection of Buprenorphine at 0.01mg/kg once a day for two days.

8. Euthanasia: If required because of poor health or pain: an animal would be temporarily immobilized against the front of the cage by a squeeze back mechanism, and anesthetized by intramuscluar injection of ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular), and then euthanized by an IV overdose (1 ml per 5kg body weight) of sodium pentobarbital (Beuthanasia). Death would be defined by stoppage of the heart as determined by a qualified and experienced person using a stethoscope to monitor heart sounds from the chest area. A necropsy as required would then be performed, again by a qualified and experienced person.

9. General Perfusion: At the end of **each** animal experiment, each monkey will be sacrificed by perfusion, and tissues obtained at necropsy for quantitative in situ studies to assess the impact of inflammation and Rx on the number and type of infected cells, burst size, etc. in cervical, vaginal and lymphatic tissues. Animals will be deeply anesthetize using Na Pentobarbital IV (25 mg/kg or until deep sedation occurs. Perfusion is required to clear the cervical and vaginal tissue samples of any blood cells.

PERFUSION METHOD-Outline

Sacrifice and perfuse (Pathology routinely performs this procedure)

7. Deprive monkey of food (not water) from afternoon prior to sacrifice
b) Inject with ketamine (15 mg/kg) 10 minutes before sacrifice
c) Inject sodium pentobarbital IV into brachial vein
d) Open chest (upper body perfusion only)
e) Clamp descending aorta
7. Cut right atrium
8. Insert needle into left ventricle, start perfusion (keep mouth open during perfusion)
9. Wash out with heparinized PBS; quantity = 1 Liter. Infuse total volume at rate of 75 ml/min (pump set to 12)

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

Monkey deprived of food (not water) from afternoon prior to sacrifice.

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.