SIV 365

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)

Experiment I.

To obtain baseline data regarding soluble inflammatory mediators in the vaginal secretion as a function of menstrual cycle and Depo Provera treatment. We will include 8 animals in this experiment. We will collect mucosal secretion samples by the wick cell method as described below **three times** weekly for two consecutive months **2-3 days** apart. At each mucosal sample collection we will draw 2-6 ml blood to determine serum reproductive hormone levels. After this we will treat the animals with Depo-Provera for two months and collect mucosal secretion samples by the wick cell method twice weekly for two consecutive months 3-4 days apart. Simultaneously we will draw 2-6 ml blood to determine reproductive hormone levels as well. After finishing this experiment the animals will participate in experiment IV.

Experiment II.

Experimental arm A

Two Herpes B seronegative female cynomolgous macaques will be treated with a bolus of progestin to render them susceptible to vaginal herpes simplex 2 infection. We will obtain 1 biopsy (described below) from the lower reproductive tract (vaginal wall or cervix) to establish baseline values of experimental parameters (outlined below). We will inoculate the pretreated animals with 10^5 plaqueforming units (PFU) of HSV-2 TK - intravaginally in less than 1 ml volume of sterile tissue culture medium. Subsequently, every two days we will inspect the vaginal lumen, cervix and the vaginal wall for signs of inflammation (epithelial redness) and lesions with a fiberoptic scope. We will collect mucosal secretion by the wick cell method. We will collect 2-8 ml blood samples to determine reproductive hormone levels during the week of treatment **at three occasions 2-3 days apart**. We will obtain 1 biopsy (described below) from a visible inflammatory lesion to perform immunohistochemical analysis with regards to SIV target cell frequency (CCR5+ or CXCR4+CD4+ T cells, CCR5+CD4+ macrophages), and expression of adhesion receptors on epithelial cells. We will perform vaginal washes with 2 times 3 ml PBS to monitor viral clearance. Mice clear this virus within 10-14 days after the intravaginal challenge. We anticipate similar results with cynomolgous macaques as well. To increase our database we will infect two more animals. We may change the infectious dose of the viral inoculum depending on our results in the first two animals.

Experimental arm B

Since macaques are relatively resistant to the formation of herpetic lesions, there is a possibility that we will not find inflammatory foci. To increase our probability of success to induce transient inflammatory foci of the female reproductive tract we have devised an alternate approach as well. We will apply nonoxynol-9 (FDA approved contraceptive agent) containing crème on the animals' vaginal and cervical mucosa for up to 5 consecutive days. Before every application we will inspect the treated area for signs of inflammation. i.e. epithelial induration and will collect mucosal secretion by the wick cell method. We will collect 2-8 ml blood samples to determine reproductive hormone levels during the week of treatment at **three** occasions **2-3** days apart. We will stop Nonoxynol-9 application as soon as visible lesion forms. Again, we will obtain 1 biopsy from the lesion perform immunhistochemical analysis with regards to SIV target cell frequency, T cell homing and epithelial adhesion receptors. We anticipate the healing of epithelial lesions within two weeks after terminating Nonoxynol-9 applications. To accelerate the healing of epithelial tissue we will employ anti-inflammatory medication as per the veterinarian's recommendation.

Experiment III.

We will induce transient inflammatory foci in the lower female reproductive tract of 4 new cynomolgous macaques employing the method that proves superior in experiment II. At the peak of inflammation we will inoculate the monkeys with autolog CD4+ T cells infected in vitro with SIVmac239. To promote the viral transmission efficiency at the time of viral inoculation animals will be sedated for 45 minutes.

Before every inoculation we will collect mucosal secretion by the wick cell method. And will collect 2-8 ml blood samples to determine reproductive hormone levels . We will place the inoculum on the surface of the inflammatory foci in 200ul volume of sterile media. To increase the probability of infection we will repeat the inoculation two more times on the following 2 days once

each day. SIV infection status will be assessed by viral load quantification on plasma obtained from weekly blood draws. After the first 4 weeks of infection, animals' SIV viral loads will be monitored biweekly until 12 weeks post-infection. From week 12 post-infection we will do monthly blood sampling until euthanasia. Progression of SIV-induced immunodeficiency disease of these animals will be monitored by the following parameters: Chem-19 panel (monthly), CBC, CD4+ count and plasma viral load at every blood draw. All blood draws and inoculations will be performed under sedation. We will follow SOP 4.01 and will stay under the maximum blood draw limits.

Control animals:

We will inoculate CD4+ T cells derived from SIVmac239-infected animals in the vaginal canal of 4 animals in 200ul volume of sterile media. To increase the probability of infection we will repeat the inoculation two more times on the following 2 days once each day. To promote the viral transmission efficiency at the time of viral inoculation animals will be sedated for 45 minutes. Before every inoculation we will collect mucosal secretion by the wick cell method. And will collect 2-8 ml blood samples to determine reproductive hormone levels . SIV infection status will be assessed by viral load quantification on plasma obtained from weekly blood draws. After the first 4 weeks of infection, animals' SIV viral loads will be monitored biweekly until week 12 post-infection. From week 12 post-infection we will do monthly blood sampling until euthanasia. Progression of SIV-induced immunodeficiency disease of these animals will be monitored by the following parameters: Chem-19 panel (monthly), CBC, CD4+ count and plasma viral load at every blood draw. All blood draws and inoculations will be performed under sedation.

Experiment IV.

To determine whether an active ulcerative inflammation in the female genital tract will lower the threshold of inoculation viral dose to achieve persistent SIV infection.

This experiment will include 12 animals. 6 controls with no genital ulcerative inflammation and 6 animals with genital ulcerative inflammation. We will induce transient inflammatory foci in the lower female reproductive tract employing the method that proves superior in experiment II. 2 animals from both of the control and treated group will be inoculated with $2x10^5$ TCID50, 2 animals from each groups will be inoculated with $2x10^4$ TCID50, and 2 animals from each goups will be inoculated with $2x10^3$ TCID50 SIVmac239 intravaginally. 6 of the animals will be the same ones that participate in experiment I. We will include an additional 6 animals to have a total of twelve animals in this group. The volume of the viral inoculum will be < 5ml, inoculation will happen only once at around the peak of inflammation of the animals with genital ulcers. To promote the viral transmission efficiency at the time of viral inoculation animals will be sedated for 45 minutes.

Before every inoculation we will collect mucosal secretion by the wick cell method, and will collect 2-8 ml blood samples to determine reproductive hormone levels . SIV infection status will be assessed by viral load quantification on plasma obtained from weekly blood draws. After the first 4 weeks of infection, animals' SIV viral loads will be monitored biweekly until week 12 post-infection. From week 12 post-infection we will do monthly blood sampling until euthanasia. Progression of SIV-induced immunodeficiency disease of these animals will be monitored by the following parameters: Chem-19 panel (monthly), CBC, CD4+ count and plasma viral load at every blood draw. All blood draws and inoculations will be performed under sedation.

Procedures

1. Depo Provera administration: The animals will be injected i.m. with 30 mg contraceptive DepoProvera . DepoProvera is available at two different concentrations. We would prefer to use the pre-filled syringes, in which it is at 150mg/ml, therefore a single dose is 200 microliters. Otherwise it is also available at 400 mg/ml, which would be a single dose of 75 microliters. The effect of 1 dose of DepoProvera lasts 30 days. We estimate that a maximum of five consecutive doses of DepoProvera will provide us a long enough time interval to achieve our experimental goals.
1. Herpes simplex-2 thymidine kinase mutant virus: This is a non-neurotropic mutant variant of Herpes simplex-2. In mice this virus does not cause lethal disease as opposed to the wild type virus. There are no published studies of the effect of HSV-2 TK- in macaques, but since it is non-neurotropic we do not expect the occurrence of lethal disease in macaques either, just as in mice.
1. Vaginal secretion collection: We collect mucosal secretion samples 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 into the vaginal cavity. The tip of the sponge will be placed on the mucosal surface and secretion will be allowed to get adsorbed for 5 minutes. After 5 minutes the sponge will be removed from the animal. We will collect maximum 3 samples simultaneously at each occasion. This method has been established for human subjects without any apparent adverse effect.
1. Vaginal wash: HSV-2 TK- virus clearance will be monitored via plaque assay on Vero cells using vaginal wash samples. We will collect mucosal secretion samples by lavage from the vaginal lumen. The posterior of the animal will be elevated and 3 ml of sterile saline or sterile phosphate buffered saline will be used to irrigate the vaginal vault non-traumatically, using flexible tubing connected to a needleless syringe. With the same syringe and tubing in place, we will collect the installed

fluid. It is important to avoid any trauma to the vaginal tissue that could result in contamination of the wash with blood. The procedure will be repeated 1 more time using another 3 ml of sterile saline or sterile phosphate buffered saline.

1. Pinch biopsies of the lower female reproductive tract: in order to determine the cellular composition of the inflammatory foci we will obtain punch biopsy samples. Samples will be obtained with a baby Tischler punch biopsy device. 2x2x2 mm tissue will be taken at the peak of inflammation. The interval between biopsies is at least 1 month and samples will be taken from 1 site at each time. We will take biopsies no more than 5 different occasions from one animal. We will take biopsies only from animals included in experiment I. Animals will be provided the following treatment for analgesics: **0.01-0.03 mg/kg buprenorphine or other analgesia administered i.m. after the procedure as recommended by the veterinarian.**

1. Blood draw: The amount of blood obtained from each of the draws will be based on the WPRC blood volume calculations (Bodyweight of animal (kg) x 60 x .05= Maximal volume of blood in mls/week, or bodyweight of animal (kg) x 60 x .20= Maximal volume of blood in mls/month. Blood draws will not exceed 10% of total blood volume at any given time, and no more than 20% of estimated circulating blood volume will be collected in a 30-day period. **Periodically** CBC will be analyzed to provide a hematology profile. If anemia is apparent, blood draw requests will be reduced and iron supplements provided (at the veterinarian's request).

1. Sedation of animals: macaques will be anesthetized using ketamine (up to 15mg/kg i.m.) or ketamine/medetomidine (5mg/kg and 30ug/kg respectively) i.m. followed by reversal with 150ug/kg atipamezole i.m. or i.v. or a more refined anesthetic regimen at the discretion of the veterinarian present.

b) Do any animals undergo any type of restraint beyond normal housing methods?
YES

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

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Viral challenge, blood draws and mucosal secretion sampling will be accomplished on animals chemically restrained by using ketamine (up to 15mg/kg i.m.) or ketamine/medetomidine (5mg/kg and 30ug/kg respectively) i.m. followed by reversal with 150ug/kg atipamezole i.m. or i.v. or a more refined anesthetic regimen at the discretion of the veterinarian present. The length of restraint is no longer than 45 minutes. The policy of chemically restraining every animal that is exposed to SIV/HIV viruses was introduced to protect personnel that are involved in animal handling during experimental procedures. These viruses induce immunodeficiency disease, which can result in uncontrolled growth of human pathogens in the infected animals.

c) Are any animals subjected to fluid or food restriction? **YES** Animals will be deprived of morning food before chemical restraint. The period of deprivation will not be longer than 4 hours.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.)

YES

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 another.