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676

20%

## 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 for toxicity testing:

4 animals will be inoculated intrarectally with the mutated TSST-1 (Q136A) at two doses in 1 ml of total volume (0.3 mg, 3.0 mg) comparable on a per kg basis to those given to rabbits that were without toxic effects and did not exceed the LD50 in rabbits. We have done this procedure before in the rhesus macaque and have not observed distress in any animals in the past. The veterinarian will administer the mutated TSST-1 and will remain with the animal for at least one hour after the procedure to monitor potential adverse effects. These animals will be monitored clinically for at least two days for signs of toxic shock such as fever and hypotension (pallor, feeble femoral pulse) and will have (6 ml) blood samples drawn before and after administration of Q136 for platelet counts (in TSS the platelet count decreases). Typically toxic shock is an acute condition, occurring within 4-8 hours of TSST-1 infusion, therefore animals will be monitored hourly for the first 4-8 hours after TSST-1 infusion and 3-4 times daily until the end of the two day period.

In addition on Day 0, 5 ml EDTA blood will be taken for baseline cell freezing.

# of Animals	Treatment	Blood draws (6 ml)
2	i.r. 0.3 mg Q136A	day -7, 0, 1, 2, 3
2	i.r. 3 mg Q136A	day -7, 0, 1, 2, 3

### 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.

On 04/23/2001-05/02/2001 we performed the toxicity testing on two animals at 0.3 mg/ml concentration, [REDACTED]. We found no adverse effect as judged by the physical well being and platelet counts of the animals.

For the second part of the project:

Four rhesus macaques will be immunized with SIVgag,env and tat antigens (30 µg each) +Q136 (0.3 mg) via the intrarectal route. Four control animals will receive Q136A alone and four animals will receive PBS only. We will monitor antibodies (IgG and IgA) in serum and intestinal secretions. Intestinal antibodies will be obtained by intestinal lavage (below). Blood will be drawn before immunization and will follow the outlined schedule:

### Collection of intestinal lavage

Macaques were placed in sternal recumbancy. Rectal mucosal lavages will be performed by gently flushing and aspirating 1.0 ml sterile saline into the rectal canal using a sterile, polypropylene 1.0ml Pasteur pipette or tuberculin syringe. SIV env-specific mucosal IgA/IgG end-point titers will be measured by ELISA.

Peripheral blood will be tested for specific antibodies against SIV Env and Gag antigens and Gag specific cytotoxic lymphocytes. In case of positive results the animals will be challenged by 10000 TCID50 SIVmac251 intrarectally. The actual volume of the virus inoculum varies, depending on the virus titer of the stock. Usually it is <5 ml. In case of negative results the immunization schedule will be repeated as described above, and the peripheral blood will again be tested for specific antibodies against SIV Env and Gag antigens and Gag specific cytotoxic lymphocytes. Prior viral challenge we will collect blood, intestinal and vaginal lavage in order to test for specific immunological parameters.

On 05/24/2001-06/28/2001 we immunized two macaques intrarectally with [REDACTED] 30 µg SIV antigens in 1 ml PBS and found no positive immune responses. Therefore we intend to change our previously approved experimental dosing in the following way:

Three rhesus macaques will be immunized with SIVgag, and env antigens (30 µg each) + [REDACTED] via the intranasal route and three rhesus macaques will be immunized with SIVgag, and env antigens (30 µg each) [REDACTED]

