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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)

Challenge with Live SIV

Macaques will be challenged with live SIV. Challenges will be performed under ketamine anesthesia (15mg/kg i.m.) by the intravenous (i.v.) route. Intravenous challenges will be performed by delivering 100 - 500 TCID₅₀ in a volume of 1 ml to a superficial vein. One TCID₅₀ stand for Tissue Culture Infectious Dose 50, which is the dose required to infect 50% of cells in tissue culture. SIV challenges will be performed in the SIV isolation center at the primate center, where animals will remain until euthanized (see above).

Lymph Node Biopsies

In addition to blood draws, we will perform lymph node biopsies from SIV infected macaques to assess the induction of immune responses in the lymph nodes. The monkey will be anesthetized with ketamine hydrochloride (20 mg/kg i.m.). 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 after 10-14 days if nonabsorbable skin sutures are placed. Topical antibiotic cream is used as needed as per vet. No more than two biopsies are collected from any one subject. The interval between biopsies is at least one month and the second biopsy is at a distinct site. The first biopsy will be the right inguinal and/or axillary lymph node, and the second biopsy will be the left inguinal and/or axillary lymph node. We will collect from a single biopsy site in a single procedure.

Biopsies of the vagina and sigmoid colon

In order to determine the cellular composition, function, and antigen specificity of cells derived from mucosal immune compartments within the vagina and sigmoid colon, we will obtain punch biopsy samples. The monkeys will be anesthetized with ketamine hydrochloride at twice the normal restraint dose (20 mg/kg i.m.) to increase its analgesic properties. Biopsies will be taken from ten different sites of colon by a fiber optic flexible pediatric gastroscope equipped with biopsy forceps. Size of biopsies will be approximately 2x2x2 mm. We also intend to take punch biopsies from two different sites of the vagina by a baby Tischler punch biopsy device, which collects a slightly larger amount of tissue, 3x3x3 mm. Punch biopsies will be performed four times from three different anatomical sites of an animal before any infection and/or treatment to assess the variability of the samples for individual animals. The interval between biopsies is at least one month and the biopsies will be taken from different sites at each time. Post-operative analgesics: 0.01 - 0.03 mg/kg buprenorphine administered i.m. 0, 12, 24, and 36 hours after the procedure will be provided as recommended by the veterinarian.