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

Naive animals will be challenged with live SIV. Challenges will be performed under ketamine anesthesia (15mg/kg i.m.) by intrarectal (i.r) route. Intrarectal challenges will be performed by delivering 1000 TCID50 SIV to the rectal mucosa using a ten cm long feeding tube or tuberculin (Tb) syringe. SIV challenges will be performed at the Biotron where animals will remain until euthanized. The SIV used in these experiments is SIVmac239-hifi. This is a cloned virus derived from SIVmac239, in which the reverse transcriptase has been modified so that it is high fidelity. It is not expected that these modifications will pose any additional hazard to either the animals or to staff. The reverse transcriptase necessary for transcribing retroviral RNA to DNA is known to be very error prone in all retroviruses, including HIV and SIV. This is this property that allows the virus to engender a large number of mutants, some of which evade the immune response (see previous project). In this project the reverse transcriptase will be "high-fidelity", that is much less prone to making these error. Therefore, these viruses would be expected to produce fewer mutants and be less able to evade the immune response. This project is being done in collaboration with Steve Dewhurst at U of Rochester, in New York. He is the chair of their IBC and feels that these viruses with mutated reverse transcriptases (RTs) are VERY unlikely to be more pathogenic than wild-type. They may, quite possibly, be LESS pathogenic than wild-type. Our whole project is based on the idea that the less error-prone RT mutant will result in a virus recombinant that is less able to generate new viral mutants, meaning that the final recombinant virus will be less able to evade host immune pressure. Thus, we would expect the virus to have no enhanced pathogenicity and quite possibly reduced pathogenicity. In terms of infection of humans, there is no reason to expect that the virus would be able to infect humans any more efficiently than the native mac239 virus. We have intentionally made no changes which influence the viral envelope or viral tropism (envelope, LTR). Thus, we can reasonably expect no change in the host range, cell tropism and species preference of the modified virus.

Blood Draws

The amount of blood obtained from each of these draws will be based on the WRPRC blood volume calculations (Bodyweight of animal (kg) x 65 x .10= Maximal volume of blood to be drawn at one time (in 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. Routine CBC's (during periods where more than one large blood draw (14mls) is requested per month, and following SIV infection) will be analyzed to provide a hematology profile. If anemia is apparent, blood draw request will be reduced and iron supplements provided (at the veterinarian's request). These blood draws are required to allow us to monitor cellular immune responses (both CTL and T helper responses) in vaccinated and SIV-infected animals and to measure the viral load in SIV-infected animals. If any animal shows an adverse hematology profile the blood draws will be put on hold until the CBC's are back to normal. The actual volumes of blood drawn on a particular week may vary due to the number of tests

which will be performed on one blood sample. These tests include virus RNA quantification and tetramer analysis at weeks 0,1,2,3 and 4; generation of CTL lines at weeks 2,4,8 and 12; and analysis of viral genetic variation at weeks 4,8,12 and 18. At these same time points, we may also opt to perform ICS **(intracellular cytokine staining)** analysis on the lymphocytes to analyze the complete immune response. The actual weeks of ICS may vary, depending on the animal's response to this virus. In all cases, the blood drawn will be less than the volumes allowed under WRPRC guidelines.

Summary of Animal Treatments:

Group	SIV	route	# animals	MHC	Blood draws (at weeks)
1	SIVmac239-hifi	i.r.	4	A*01+	0,1,2,3,4,8,12,18
2	SIVmac239-hifi	i.r.	4	A*01-	0,1,2,3,4,8,12,18

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

Animals will only be food restricted (fasted) the night before a procedure, but not for any other reason.

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.

There is a very limited amount of space designated for SIV-specific research at the Biotron. The cages at the Biotron are for animals up to 10 kg. Most of the animals used are less than 10 kg for the entire project; however, due to the variable response to SIV infection, some animals may live long enough to grow in weight to over 10 kg. Also, due to limited availability of rhesus macaques at the center, we may have to accept animals over 10 kg at the beginning of a study. Due to space limitations, we request an exemption from the minimum space requirements at Biotron for animals on SIV-specific research that exceed 10 kg. We plan to relocate SIV rooms in the new addition to the annex which will solve the problem.